BIOCHEMICAL AND GENETIC ANALYSES

OF LAVENDER-PURPLE SPATHES IN ANTHURIUMS

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ABSTRACT

Systems controlling lavender-purple spathe color in anthuriums were investigated biochemically and genetically. Regression analysis of biochemical data showed that the blueness of anthurium spathe color was the result of cyanidin 3-rutinoside and its interaction with cell sap pH and major flavonoids from A. amnicola, A. andraeanum and A. antioquense. The ratio of cyanidin 3-rutinoside to acacetin C-diglycoside was found to be the most important factor determining the degree of blueness in the regression model. Results from in vitro copigmentation experiments suggested that the inhibition effect of acacetin C-diglycoside may be due to some unknown factors strongly associated with the flavone.

A. amnicola and A. antioquense of section Porphyrochitonium, and A. andraeanum and A. formosum of section Calomystrium were shown to be closely related based on their crossability and on the meiotic behavior of hybrids. All interspecific hybrids showed 15 bivalents at metaphase I; however, some hybrids showed spindle abnormalities. Interspecific progenies of A. amnicola produced the lowest percentage of viable pollen.

A genetic scheme controlling lavender-purple spathe color was proposed. The system consisted of two loci controlling red-orange-white colors and a recessive epistatic locus for the copigmentation inhibitor. General combining ability of parental species for the quantity of the factors involved in color expression was determined.

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INTRODUCTION

Anthurium is one of the largest genera in the family Araceae, consisting of more than 700 species. Its members are native to tropical America with the distribution extending from Central Mexico and West Indies to Central South America (Croat, 1983).

From the horticultural standpoint Anthurium andraeanum Hort. is the most important member of the genus. It is cultivated as a cut-flower crop in the tropics and some European countries. Another important species is A. scherzerianum Schott grown as a flowering potted plant in temperate areas. Other species cultivated as ornamental plants are A. hookeri Kunth, A. scandens Engler, A. trinerve Miquel and A. warocqueanum Moore.

The first anthurium brought to Hawaii from London in 1889 had a shell-pink spathe (Neal, 1965). Now, however there is a multitude of spathe colors including white, coral, orange, pink and red. The standard cut-flower cultivars have spathes that are similar to those of A. andraeanum. Novelty cultivars recently released by the University of Hawaii, such as 'Calypso' and

'Trinidad', have upright spadices and spathes and are referred to as tulip-type (Kamemoto, 1981).

The discovery of A. amnicola, a miniature species with lavender spathes by Dressler (1978) has stimulated an interest in hybridization for the development of purple anthuriums. Several interspecific hybrids have been made with this species. In general, the first generation hybrid does not produce lavender spathes. To date, only A. antioquiense and A. formosum crossed to A. amnicola have yielded purple-spathe offspring. Lavender to purple spathes are obtained mostly from backcrosses to A. amnicola. However, their spathes are small. Recently, fairly large purple spathes have been obtained from hybrids involving A. amnicola and/or A. formosum.

Biochemical analyses of A. amnicola and its hybrids had suggested that the concentration of cyanidin 3-rutinoside, the presence of flavonol C-monoglycoside, and the pH of tissues might be important in color determination of spathes (Marutani, 1984).

The present study was aimed to elucidate the systems affecting lavender-purple color in anthuriums and to determine the possible use of interspecific hybrids for further improvement of purple anthuriums.

II. LITERATURE REVIEW

2.1 Anthurium Taxonomy and Genetics

2.1.1 Taxonomy

The genus Anthurium, one of the largest and most taxonomically complex genera in the family Araceae, comprises more than 700 species (Sheffer and Croat, 1983). The members are either herbaceous or climbing perennials. This genus can be identified by the presence of the spathe and spadix, firm leaves with primary lateral veins mostly connected by a well-defined vein running inside and parallel to the margin (antemarginal vein), small reticulate veins, and berries which are often colored and showy (Bailey Hortorium, 1976).

Anthurium was first subgenerically classified by H. W. Schott in 1860. In 1905, Engler classified the genus into 18 sections based primarily on the number of ovules per locule, leaf shape and texture, spathe and spadix shapes, and berry shape. Using the major characters of Engler's classification system, Sheffer and Kamemoto (1976b) constructed six groups. Cross compatibility among 57 species confirmed the distinction of all groups except group V and VI which are closely related. Recently, Croat and Sheffer (1983) modified

Engler's classification leading to the rearrangement of several species. The system was based primarily on leaf shape and venation, stem size and shape, and number of ovules per locule.

2.1.2 Cytogenetics

Chromosome numbers of Anthurium were first reported by Campbell (1905). In 1983, Sheffer and Croat thoroughly reviewed the previous chromosome counts and added some new counts. The somatic chromosome numbers in 146 species varied from 20 to ca. 124, with the most common number of 30.

Sheffer and Kamemoto (1976a) proposed four polyploid series of 20-40, 24-30-48-84, 28-56 and 30-60-90-ca. 124. The basic numbers of 5, 6 and 7 were suggested. They considered n=15, previously proposed by Gaiser (1927), as a secondary basic number.

Somatic chromosome numbers of 30 and 32 have been recorded for Anthurium andraeanum. It has been suggested that the two extra chromosomes in the 2n=32 counts were possibly loose satellites (Sheffer and Kamemoto, 1976a) or B chromosomes (Kaneko and Kamemoto, 1978). Kaneko and Kamemoto (1978) reported meiotic irregularities in A. andraeanum 'Kaumana' and 'Uniwai'.

They suggested that the irregularity indicated a hybrid origin of cultivated anthuriums. However, a recent study by Marutani (1984) showed normal meiotic pairing at prometaphase I in both cultivars and precocious separation of one pair leading to the formation of micronuclei in 'Kaumana'.

Marutani (1984) studied meiotic configurations of A. amnicola, A. andraeanum and closely related taxa. All species and their hybrids showed 15 pairs of chromosomes at prometaphase I. However, pollen fertility of hybrids was greatly reduced, especially in intergroup hybrids. Interestingly, pollen stainability of hybrids between species that belonged to group VI and A. amnicola of group I were in the same range as interspecific hybrids within group VI.

B chromosomes have been reported to be present in Anthurium, and are especially widespread in the section Cardiolonchium. The number ranges from 1 to 4 (Sheffer and Croat, 1983). In A. warocqueanum, which has two or more B chromosomes, two B chromosomes tend to pair and divide normally in meiosis. The univalent B chromosomes lag, form micronuclei and are eventually eliminated. Plants with more than 4 B chromosomes exhibit reduction of A chromosome pairing (Marutani and Kamemoto, 1983).

This may account for the number of B chromosomes in the natural populations of Anthurium being limited to four.

Sheffer (1974) reported inheritance of B chromosomes in 7 crosses to be irregular. The effective inheritance of B chromosomes was suggested to be non-random. Accumulation of the chromosomes was not observed. Later studies confirmed the non-random inheritance of B chromosomes (Marutani and Kamemoto, 1983). In selfed progenies of A. warocqueanum containing three B chromosomes the highest frequency was two B chromosomes instead of three as expected if the chromosomes were distributed randomly.

2.1.3 Interspecific hybridization

Hybridization among species of Anthurium was reported as early as 1905 by Engler. The hybrids were offspring of A. andraeanum with A. cerrocampanense, A. lindenianum, A. magnificum, A. nymphaeifolium, A. ornatum, A. splendinum, A. veitchii, A. warocqueanum and with a hybrid of A. garagaranum and A. nymphaeifolium.

In the anthurium breeding program at the University of Hawaii, extensive interspecific hybridization has been carried out to study species relationships and to improve

flower quality. Sheffer and Kamemoto (1976b) attempted interspecific hybridization among 56 species to study species relationships. Higher crossability in intragroup than intergroup pollination was reported, suggesting close relationship of species within each group. In 1977, Sheffer and Kamemoto tried to incorporate the attractive leaves into the showy flower species by making intergroup pollination between A. andraeanum and species with velvety leaves. However, their attempts were unsuccessful. Later, a flowering potted plant, A. scherzerianum, and a foliage species, A. wendlingerii, were successfully hybridized. The hybrids were fertile and had showy spathes (Kamemoto and Sheffer, 1978).

A. amnicola has been used recently in the hybridization program of the University of Hawaii to improve lavender-purple anthuriums. As many as five species have been incorporated into a hybrid.

2.1.4 Color inheritance

Studies on the inheritance of spathe colors have been conducted at the University of Hawaii for over three decades. Based on their early work, Kamemoto and Nakasone (1955, 1963) reported that white and orange bred true, and red x red produced all red or red and orange in

the ratio of 1:1. They concluded that a multiple allele system was operating in which red (R^r) was dominant to orange (R^o).

Studies on interspecific hybrids of A. andraeanum and closely related taxa indicated that the inheritance of both spadix and spathe colors were controlled by multigenic systems (Sheffer and Kamemoto, 1977). Qualitative and quantitative analyses of pigments were performed to define the genetic system controlling spathe colors (Iwata, 1980). A system of monogenic control for each major anthocyanin was proposed. The gene M controlled cyanin production and the gene O controlled pelargonin production. The O locus was suggested to show recessive epistasis over the M locus. The proposed genotypes were M O for the red group (red and pink spathes), mmO for the orange group (orange and coral spathes), and oo for the white. Kamemoto et al. (in press) suggested that the genotypes MMOO, MMOo and MmOO are responsible for red spathes. The genotypes MmOo, mmOo, mmOO and oo are responsible for pink, coral, orange and white spathes, respectively.

2.2 Anthocyanins

2.2.1 Anthocyanins and flower colors

Anthocyanins are a class of flavonoids. In nature, they always exist in glycosidal forms. They are water-soluble pigments responsible for most scarlet, orange, red, purple and blue colors in various plant organs. Colors of the anthocyanins are affected by the nature and the concentration of the pigments, the presence of copigments, and the pH and the metal ion content in the vacuole sap (Harborne, 1967).

Anthocyanins can be categorized into three main groups by the number of hydroxyl groups in the B ring. The greater the number of hydroxyl groups the bluer is the color. Pelargonins having one hydroxyl group are orange-red; cyanins having two hydroxyl groups are red-magenta; and delphinins having three hydroxyl groups are purple-blue. Methylation of the hydroxyl groups makes the anthocyanins become redder (Asen, 1976; Harborne, 1976; Osawa, 1982).

The concentration of anthocyanins affects both the value (intensity) and the hue (shade) of colors. A difference in cyanin concentration has been found to be responsible for a difference between the pink and red flowers (Yasuda, 1971; Iwata, 1980). In roses, the color

of the petals changed from purple to red as the concentration of cyanins increased (Biran et al., 1974b).

Copigmentation, a phenomenon wherein anthocyanins interact with themselves or other organic compounds, makes the colors of the solution brighter, bluer or more stable (Osawa, 1982). Intermolecular copigments play an important role in bathochromic shift (absorption maximum shifts toward higher wavelength), especially in the visible range (Brouillard, 1983). Asen et al. (1971b) found that the orange sport of 'Red Wing' azalea lacked the copigments which were present in the normal plant.

The degree of bathochromic shift caused by copigmentation depends on the type of anthocyanin (Asen et al., 1972; Yazaki, 1976), the amount of anthocyanin (Asen et al., 1972) and the anthocyanin-copigment ratio (Asen et al., 1971b; Asen et al., 1972; de Loose, 1978; Hoshino et al., 1980; Yazaki, 1976).

Metal ions are occasionally important for blue complex formation. Iron was found to be an essential factor in blue-color formation in roses (Yasuda, 1971; Yasuda, 1982), while calcium and magnesium were found in blue pigment complexes in other plants (Ishikura and Takahama, 1972). These complexes involving metal ions are affected by the pH of the cell and the nature of the

organic acids in the buffering system of the cell. When metal ions form chelates, they have higher affinity with some organic acids than with anthocyanins (Bayer et al., 1966; Jurd and Asen, 1966)

Anthocyanins can reversibly transform their structure with pH change. Increasing pH will lead to a bathochromic shift, while decreasing pH will lead to a hypsochromic shift (Brouillard, 1983; Chen and Hrazdiza, 1981). The pH change seems to be the major factor in the color change in aging flowers (Asen et al., 1971a). Aged anthurium spathes turned from red to blue with simultaneous increase of tissue pH, ammonium ion and phenol concentration (Paull et al. 1985). Asen et al. (1977) found that in epidermis cells of morning glory a pH shift from 6.5 to 7.5 was associated with the change in flower color from strong reddish-purple to light blue.

A survey by Stewart et al. (1975) showed an association between a high vacuolar pH value and purplish or bluish flower genera. However, they concluded that pH was only one of numerous factors determining color in the living cell since plants of the same genus but with a different flower color exhibited the same pH.

The number of pigmented layers in the flower also contributes to flower color. In poinsettia, three

mutations from red to pink bract color resulted from loss of pigments from epidermal layer (Stewart and Asen, 1966). The ability of tissue layers to reflect or transmit light is also important for color expression. In rose, the thicker the colorless mesophyll layer, the redder is the flower (Biran et al., 1974a).

2.2.2 Anthocyanins in anthurium

In the genus Anthurium, the most common anthocyanin is cyanidin 3-rutinoside (Iwata, 1980; William et al., 1981; Marutani, 1984). Robinson and Robinson (1932) first reported that the pigment in young leaves and the orange-scarlet spathe of A. scherzerianum was pelargonidin 3-pentoseglycoside. This was later identified by Harborne (1967) as pelargonidin 3-rutinoside. Forsyth and Simmonds (1954) working on A. andraeanum, reported that the red spathe contained a cyanin while the orange spathe contained both a cyanin and a pelargonin. Lowry (1972) identified the anthocyanins in A. andraeanum as cyanidin and pelargonidin 3-rutinoside. However, Iwata (1979) reported finding cyanidin and pelargonidin 3-rutinoside in pink and red spathes, but only pelargonidin 3-rutinoside in orange spathes of cultivated

A. andraeanum. Marutani et al.(1984) found peonidin 3-rutinoside as a minor anthocyanin in A. amnicola. This was a unique anthocyanin in the genus Anthurium.

2.2.3 The genetics of flower color involving anthocyanins

The inheritance of anthocyanins in flowers has been investigated in many flowering crops (Harborne, 1967; Lawrence and Price, 1940; Scott-Moncrieff, 1936; Seyffert, 1982). Scott-Moncrieff (1936) inferred that 1) the presence of more oxidized anthocyanins were dominant to less oxidized ones, 2) the presence of 3,5-diglycosidic anthocyanins was dominant to 3-mono-glycosidic ones, 3) anthocyanin and copigment production were generally dominant to their absence, and 4) low pH of cell sap was dominant to high pH.

In Dahlia variabilis, genes A and B control cyanin production and gene I controls flavone production. The function of genes A and B is partially suppressed when gene I is present. Flavone produced by gene I action can form complex with cyanin to produce purplish color (Lawrence and Scott-Moncrieff, 1935).

The genetics of petunia flower color is one of the best known systems. Major steps in the conversion pathway of the flavanone naringinin to anthocyanins are

controlled by seven loci. Genes from six other loci control anthocyanin modification (Wiering, 1974). Moreover, there are five additional loci involved in maintaining the pH of the cell sap (de Vlaming *et al.*, 1982).

To date, there are two known loci controlling anthocyanin production in anthuriums. Gene *M* controls production of cyanidin 3-rutinoside and gene *O* controls production of pelargonidin 3-rutinoside. The *O* locus has a recessive epistatic effect on the *M* locus (Iwata, 1980).

III. MATERIALS AND METHODS

3.1 Plant Material

The anthurium plants used in this study were part of the breeding program at the University of Hawaii. The major species involved in this study were Anthurium amnicola Dressler, A. antioquiense Engler, A. formosum Schott, A. andraeanum Hort. 'Marian Seefurth', A. lindenianum C. Koch & Augustin and A. kamemotoanum Croat.

A. amnicola with light lavender spathes and A. antioquiense with very pale pinkish lavender spathes are native to Colombia. Both of these closely related species were placed in section Porphyrochitonium by Croat and Sheffer (1983). A. formosum of the section Calomystrium is characterized by very large creamy spathes and thrives at high elevations in Costa Rica, Panama, Colombia and Ecuador. A. andraeanum in the section Calomystrium is a native of Colombia and Ecuador. Most of the present-day cultivars such as the pink 'Marian Seefurth' are probably of interspecific origin and might be appropriately designated as A. andraeanum Hort. A. kamemotoanum, a native of Panama, is a member of section Calomystrium. This species has a dark magenta

spathe. A. lindenianum is also a member of section Calomystrium. It has a white spathe.

Half diallel crosses were made among A. amnicola, A. antioquiense, A. formosum and 'Marian Seefurth' to investigate meiotic behavior of hybrids and to determine general combining ability for factors affecting the blueness of the spathe. The various segregating hybrid populations used in the biochemical and inheritance studies are listed in Table 1. The anthurium species and hybrids used in the regression analysis are listed in Table 2.

3.2 Biochemical Analyses

3.2.1 Color and pH measurements

Spathe color was determined by using the Royal Horticultural Society Color Chart (RHSCC). Objective measurements were obtained by using the Hunterlab colorimeter (Hunter, 1967). Hunterlab coordinate b was recorded for data analysis. The color quality parameter b indicates relative position on a blue (negative value) to yellow (positive value) scale. The b value was used as color index of purple to red spathes in this study.

Sap pH was measured by crushing a small piece of spathe with a glass rod. The pH reading was immediately

Table 1. List of interspecific hybrids utilized in biochemical and genetic analyses.

Cross number	Parents
573	A. amnicola x (A. kamemotoanum x A. formosum)
587	[(A. andraeanum x A. kamemotoanum) x A. formosum] x A. amnicola
631, 632	(A. lindenianum x A. amnicola) x A. lindenianum
634	(A. lindenianum x A. amnicola) x A. amnicola
692, 696 and 697	[(A. andraeanum x A. lindenianum) x A. amnicola)] x A. formosum
693	587-29 x A. formosum
733	(A. andraeanum x A. amnicola) x A. formosum
739, 745	587-15 x A. formosum
741*, 753	[(A. andraeanum x A. lindenianum) x A. amnicola] x A. formosum
747, 750	(A. lindenianum x A. amnicola) x (A. kamemotoanum x A. formosum)
751	(A. amnicola x A. lindenianum) x A. formosum
758*, 762	[(A. andraeanum x A. kamemotoanum) x A. formosum] x A. formosum
763	(A. andraeanum x A. antioquiense) x A. antioquiense

* These crosses were made with A. formosum (A 507) which differed from those (A290, A291) used in other crosses.

Table 2. List of anthurium species and hybrids used in statistical analysis.

Accession or cross number	Species or parents	Number of plants
H 33	*A. andraeanum* 'Marian Seefurth'	1
A 291	*A. formosum*	1
A 417	*A. amnicola*	1
A 490A	*A. antioquiense*	1
573	*A. amnicola* x (*A. kamemotoanum* x *A. formosum*)	3
587	[(*A. andraeanum* x *A. kamemotoanum*) x *A. formosum*] x *A. amnicola*	54
634	(*A. lindenianum* x *A. amnicola*) x *A. amnicola*	7
692	[(*A. andraeanum* x *A. lindenianum*) x *A. amnicola*] x *A. formosum*	10
693	587-29 x *A. formosum*	5
756	*A. amnicola* x *A. andraeanum*	26
763	(*A. andraeanum* x *A. antioquiense*) x *A. amnicola*	17
768	*A. antioquiense* x *A. andraeanum*	25

taken by using a surface combination pH electrode (Corning Science Products) with a Corning pH meter model 125.

3.2.2 Flavonoid analysis

3.2.2.1 Extraction

A piece of a newly opened spathe was weighed and homogenized in 0.1% HCl-MeOH. The suspension was kept at -20°C for at least 18 hours and then filtered with Whatman No.1 paper. The extract was dried using a rotary evaporator at 40°C. Pigments were dissolved in an aqueous solution of 6% acetone and 10% acetic acid (10 ml/gm spathe). The samples were passed through a 0.45 micron membrane filter prior to HPLC injection.

3.2.2.2 Identification and quantification

3.2.2.2.1 Anthocyanins

Anthocyanins were separated through a 10 micron Lichrosorb RP18 HPLC reverse-phase column (250 mm x 4.6 mm). The analysis was done using a modified elution profile described by Akavia and Strack (1980). An aqueous solution of 1.5% H_3PO_4 (pump A) and a mixture of 20% acetic acid and 25% acetonitrile in 1.5% H_3PO_4 (pump B) were used in the gradient program as follows:

Time (min)	% solvent from pump B
0-10	25
10-20	25-45, linear program
20-42	45-55, linear program.

The flavonoid extracts were injected at 10 minutes after the program started. The flow rate was 1 ml/min and the detector was set at 530 nm.

Anthocyanin pigments were identified by comparing the retention time with the standard pigments. Authentic standards for cyanidin 3-rutinoside and pelargonidin 3-rutinoside were isolated from bracts of Euphorbia pulcherrima 'Amy' (Asen, 1979), while the peonidin 3-rutinoside standard was isolated from Prunus avium L. 'Bing' (Lynn and Luh, 1964).

The peak area was computed by electronic integration using a Shimadzu C-R 3A Chromatopac recorder/integrator. Relative amounts of each anthocyanin were expressed by integrator area/ fresh weight (volt/ gram).

3.2.2.2.2 Other flavonoids

The flavonoid extracts were separated through a 10 micron Lichrosorb RP18 column (250 mm x 4.6 mm). The analytical method described by Vande Casteele et al. (1982) was employed with some modification.

A two-solvent system, consisting of degassed aqueous solution of 5% formic acid in pump A and absolute methanol in pump B, was used in an elution profile. The elution program was:

Time (min)	% solvent from pump B
0-11	10
11-13	10-30, linear program
13-24	30-50, linear program
24-29	50-70, linear program
29-31	70-75, linear program
31-37	80, isocratic.

The flow rate was 1.5 ml/min and the injection was made 10 minutes after the program started. Chromatograms were detected at 270 nm.

Authentic standards of major flavonoids of A. andraeanum (acacetin C-diglycoside), A. amnicola (acacetin 6-C-glucosylglycoside) and A. formosum (flavonol C-monoglycoside) were extracted from the respective species (Marutani, 1984). Identification of flavonoids was performed by comparing the retention time.

The relative amounts of each flavonoid were computed and expressed using the same method as for anthocyanins.

3.2.2.3 Isolation

Leaves were used for the isolation of the major flavonoids of A. amnicola, A. andraeanum and A. formosum. Samples were dried at 80°C for 2 days and extracted with methanol. The extracts were filtered through Whatman No.1 paper and concentrated under vacuum at 40°C. Two paper chromatographies using BAW [tertiary butanol: glacial acetic acid: water (3:1:1 by volume)] and 15% acetic acid were performed consecutively. The concentrated extracts were applied as a band on 46 cm x 54 cm Whatman 3 mm paper. The Rf values under long wave-length ultraviolet light were compared with data reported by Marutani (1984). After the first separation using BAW, the bands containing acacetin 6-C-glucosyl-glycoside (Rf 0.46), acacetin C-diglycoside (Rf 0.75) and flavonol C-monoglycoside (Rf 0.41) were separated and eluted with methanol. The eluents were then concentrated under vacuum at 40°C. The compounds were collected and applied to another paper for the second chromatography. The bands of acacetin 6-C-glucosylglycoside (Rf 0.73), acacetin C-diglycoside (Rf 0.90) and flavonol C-mono-glycoside (Rf 0.46) were cut out and eluted with methanol. The eluates were dried under vacuum at 40°C

and the compounds were collected. The relative quantity and purity of compound were examined by HPLC profile.

3.2.3 Organic acid analysis

A piece of the same spathe used in flavonoid analysis was weighed and homogenized in 80% ethanol. The suspension was stored overnight before filtering through a Whatman No.1 paper. Extracts were dried by rotary evaporator at 40°C. Organic acids were eluted with 80% ethanol (10 ml/gm fresh weight). Prior to HPLC injection, the chlorophylls were removed from samples by using Sep-pak C18 cartridges (Waters Associates).

An isocratic HPLC elution profile described by Picha (1985), was applied to separate organic acid through an ion exclusion HPX-87 column (300 mm x 7.8 mm, Bio-Rad). The system used 0.008 N H_2SO_4 with a flow rate of 0.8 ml/min. Chromatograms were detected at 210 nm.

The relative quantities of organic acids were computed and expressed using the same method as for flavonoids.

3.2.4 Tissue analysis

The spathe samples of A. amnicola, A. andraeanum, A. antioquiense, A. formosum and progenies from cross 587

were dried at 80°C for 3 days and weighed. The samples were ashed at 500°C for 6 hours prior to liquefaction with 2 N HCl. Liquid samples were subjected to an atomic absorption spectrophotometer to obtain the iron and magnesium contents.

3.2.5 Regression analysis

The objective was to construct a linear multiple-variable regression model for blueness as affected by the various biochemical compounds. PC-SAS software (SAS Institute, 1987) was used. Hunterlab's b values were used as the indicator of blueness, which was the response variable. The ratios between anthocyanins and flavonoids were transformed to natural logarithmic values since an earlier study (Asen et al., 1972) indicated that effects of copigmentation on the absorption maxima were logarithmic.

Preliminary analysis on 37 plants of cross 587, a segregating population, was performed to test the significance of the effects of spathe pH, copigmentation, organic acids and metal ions on color expression. Spathe pH, the ratio between the sum of the cyanidin 3-rutinoside and peonidin 3-rutinoside contents and the sum of the acacetin C-diglycoside, acacetin

6-C-glucosylglycoside and flavonol C-monoglycoside contents, the total organic acid, and the sum of the iron and magnesium ions were used as the regression variables. The type III (partial) analysis of the general linear model procedure (PROC GLM) was used to evaluate the significance of the variables (Freund and Littell, 1981). Only factors found to be significant were subjected to further analysis.

The biochemical factors found in red and purple spathes of six segregating populations (cross 573, 587, 634, 692, 693 and 763) were used to construct a linear multiple-variable regression model for blueness using PROC GLM. All hybrid populations segregated purple and red in a 1:1 ratio, except cross 692 which segregated purple, red and white in a 1:1:2 ratio. The spathe pH, the quantities of the anthocyanins (ie. cyanidin 3-rutinoside, peonidin 3-rutinoside and the sum of the two), and the natural logarithmic values of the nine ratios between each anthocyanin value and each of the flavonoids (acacetin C-diglycoside, acacetin 6-C-glucosylglycoside and flavonol C-monoglycoside) were used as the regression variables. Since copigmentation has been shown to be affected by both the anthocyanin concentration and the anthocyanin-flavonoid ratio (Asen

et al., 1972), only models that included the same anthocyanin that appeared in any anthocyanin-flavonoid ratios were valid. Type I (sequential analysis) F-tests were used to verify the model. Type III (partial analysis) F-tests or F-tests of the regression coefficients were used to evaluate the significance of the factors in the selected model. The model with the highest coefficient of determination (r^2) was selected and was used to derive the regression equation.

The significance of the factors in the segregating population was verified by using the PROC GLM to test them in a larger population consisting of the four species, two first-generation hybrids and the same six segregating crosses (Table 2). The model and the significance of the factors were analyzed using type I and III analyses similar to the method used to derive the regression equation.

3.2.6 Factors affecting color expression in vitro

Cyanidin 3-rutinoside, extracted from A. andraeanum 'Kaumana', was used to test the copigmentation effect of the major flavonoids from A. amnicola, A. andraeanum, A. antioquiense and A. formosum. Different combinations of cyanidin 3-rutinoside with acacetin C-diglycoside,

acacetin 6-C-glucosylglycoside, and flavonol C-monoglycoside were made with an anthocyanin: copigment ratio of 1:1 (by weight). Cyanidin 3-rutinoside was used at 3.17×10^{-3} M (2 mg/ml). The mixtures were first dissolved in methanol since acacetin C-diglycoside has poor solubility in water.

The mixtures were dried and redissolved in pH 5.66 buffer solution (0.01 M citrate-0.02 M Na_2HPO_4 mixture). Absorption spectra were recorded using a Perkin-Elmer UV-VIS spectrophotometer model 554 in the red-blue range (450-650 nm) immediately after the pigment was dissolved and 18 hours later.

Effects of pH on copigmentation were investigated by diluting the mixtures with 0.1 M citric acid to pH 3.44. Absorption spectra were recorded immediately and 18 hours later.

3.3 Genetic Analyses

3.3.1 Cytogenetic studies

3.3.1.1 Meiotic analysis

Young spadices were fixed in a modified Carnoy's solution [chloroform: 95% ethanol: glacial acetic acid (1:1:2) by volume] for at least 24 hours at -20°C before the study. Anthers were removed from the spadices and

squashed in 45% acetic acid. Pollen mother cells were stained with 1% aceto-carmine. Semi-permanent slides were made by mixing Hoyer's solution with the PMC suspension in a 1:1 ratio.

The meiotic cells were examined at metaphase I under a phase-contrast microscope. Photographs of the chromosomes were taken using Kodak Technical Pan film (at ASA 125). Film was developed with D-19 solution and the negatives were printed on Kodak Polycontrast paper (at the equivalent of grade 4 paper).

3.3.1.2 Fertility study

Anthers were collected in gelatin capsules and transported from the greenhouse. The pollen grains were stained with 1% aceto-carmine for 5-15 minutes. The percentage of pollen stainability was used as the pollen fertility index. One thousand pollen grains per spadix were counted.

3.3.2 Inheritance study

The color on the adaxial side of the spathe was recorded from the offspring of segregating crosses. The color was subjectively classified into three discrete color groups of white, purple and red. The parental

species were assumed to be homozygous at the loci affecting the lavender-purple spathe color. Chi-square tests were performed to test goodness of fit to expected phenotypic ratios.

3.3.3 General combining ability test

Data on regression factors determining the lavender-purple spathe color from half-diallel populations were used to compute the general combining ability (GCA). The GCAs were calculated using a formula given by Falconer (1981). The formula was:

$$G_a = [T_a/n-2] - (T_n)/\ n(n-2)$$

where: G_a = GCA of the parent A

T_a = Summation of all progenies of the parent A

T_n = Total summation of all progenies of the parent N

n = the total number of the parents.

IV. RESULTS AND DISCUSSION

4.1 Biochemistry of Pigmentation

4.1.1 Analysis of factors affecting lavender-purple color

The results of the preliminary analysis of cross 587 showed that spathe pH and the ratio between total anthocyanins and total flavonoids were the significant factors at the 0.15% level in the Type III analysis (Table A1). Organic acid content and the amount of iron and magnesium ions were found to be nonsignificant. Thus, analyses for organic acids and metal ions were excluded from further analyses.

In the segregating crosses 573, 587, 634, 692, 693 and 763, the effects of copigmentation and pH on color expression were analyzed. Spathe pH, the amounts of anthocyanins that can form copigmentation complexes (ie. cyanidin 3-rutinoside, peonidin 3-rutinoside and their total) and their ratios with the three flavonoids (ie. acacetin C-diglycoside, acacetin 6-C-glucosylglycoside and flavonol C-monoglycoside) were used as regression variables to generate linear multiple-variable models with 3, 4 and 5 factors (Table 3). Only models which included both a specific anthocyanin and the ratios of the same anthocyanin with the copigments were considered.

The coefficient of determination of the model (r^2) was increased when the number of the regression variables increased from three to four in the model. However, the best model with five factors had a lower coefficient of determination than the best of the four-factor model. Moreover, some variables in the five-factor models were not significant even though the models were significant at the 0.05 level. It was concluded that flavonol C-monoglycoside was not a contributing factor. The best models were those with 4 factors including spathe pH, either cyanidin 3-rutinoside or the total anthocyanins and their respective ratios to acacetin C-diglycoside and acacetin 6-C-glucosyl-glycoside. The very small difference between the two models may be due to the very small amount of peonidin 3-rutinoside. In addition, it should be noted that the 3-factor models which included peonidin 3-rutinoside had at least one nonsignificant factor. Thus, the model with total anthocyanin was disregarded.

The best regression model constructed for the population was derived from Table 4 as:

$$b = 56.31 + 1.42\ LCM - 2.36\ LCN - 9.77\ pH + 0.0004\ Cy3R$$

Table 3. Results from PROC GLM analyses using thirteen biochemical regression variables to determine Hunterlab's b value.

Dependent variable: Hunterlab's b
Regression variables: Spathe pH; the amounts of anthocyanins that can form copigmentation (ie. cyanidin 3-rutinoside, peonidin 3-rutinoside and the sum of the two); the natural logarithmic values of their ratios with each of three flavonoids (ie. acacetin C-diglycoside, acacetin 6-C-glucosyl-glycoside and flavonol C-monoglycoside).

Source[a]	r^2	Pr>F
Models with three factors:		
pH^{ns} $Cy3R^{ns}$ LCF^{ns}	0.083	0.297
pH^{*} $Cy3R^{ns}$ LCM^{ns}	0.166	0.002
pH^{**} $Cy3R^{**}$ LCN^{**}	0.331	0.000
pH^{ns} $Peo3R^{*}$ LPF^{**}	0.255	0.006
pH^{ns} $Peo3R^{**}$ LPM^{*}	0.241	0.000
pH^{**} $Peo3R^{ns}$ LPN^{ns}	0.217	0.000
pH^{*} An^{ns} LAF^{ns}	0.094	0.239
pH^{**} An^{ns} LAM^{ns}	0.157	0.004
pH^{**} An^{**} LAN^{**}	0.333	0.000
Models with four factors:		
pH^{**} $Cy3R^{**}$ LCN^{**} LCM^{*}	0.381	0.000
pH^{*} $Cy3R^{ns}$ LCN^{**} LCF^{ns}	0.293	0.009
pH^{**} An^{**} LAN^{**} LAM^{*}	0.379	0.000
pH^{**} An^{ns} LAN^{*} LAF^{ns}	0.292	0.009
Model with five factors:		
pH^{*} $Cy3R^{ns}$ LCN^{ns} LCM^{*} LCF^{ns}	0.323	0.014
pH^{*} An^{ns} LAN^{ns} LAM^{*} LAF^{ns}	0.326	0.013

^{ns},*,** Non-significant, significant at the 5% and 1% level, respectively.

[a] pH = spathe sap pH
Cy3R = cyanidin 3-rutinoside content
LCF = ln (cyanidin 3-rutinoside/ flavonol C-monoglycoside)

Table 3. (continued). Results from PROC GLM analyses using thirteen biochemical regression variables to determine Hunterlab's b value.

LCM	= ln (cyanidin 3-rutinoside/ acacetin 6-C-glucosylglycoside)
LCN	= ln (cyanidin 3-rutinoside/ acacetin C-diglycoside)
Peo3R	= peonidin 3-rutinoside content
LPF	= ln (peonidin 3-rutinoside/ flavonol C-monoglycoside content
LPM	= ln (peonidin 3-rutinoside/ acacetin 6-C-glucosylglycoside)
LPN	= ln (peonidin 3-rutinoside/ acacetin C-diglycoside)
An	= cyanidin 3-rutinoside + peonidin 3-rutinoside
LAF	= ln [(cyanidin 3-rutinoside + peonidin 3-rutinoside)/ flavonol C-monoglycoside]
LAM	= ln [(cyanidin 3-rutinoside + peonidin 3-rutinoside)/ acacetin 6-C-glucosyl-glycoside]
LAN	= ln [(cyanidin 3-rutinoside + peonidin 3-rutinoside)/ acacetin C-diglycoside]

Table 4. Regression analysis of relationships between Hunterlab's b value and four selected factors in segregating population of crosses 573, 587, 634, 692, 693 and 763.

Regression Analysis

Dependent variable: Hunterlab's b value

Parameter*	Estimate	T for HO: parameter=0	Pr>/T/	SE of estimate
Intercept	56.3137	3.31	0.0015	17.0055
LCM	1.4174	2.43	0.0175	0.5826
LCN	- 2.3582	- 4.83	0.0001	0.4882
pH	- 9.7657	- 3.22	0.0019	3.0305
Cy3R	0.0004	2.73	0.0081	0.0001

* b = Hunterlab coordinate for yellow-blue
LCM = ln (cyanidin 3-rutinoside /acacetin 6-C-glucosylglycoside)
LCN = ln (cyanidin 3-rutinoside /acacetin C-diglycoside)
pH = pH of spathe sap
Cy3R = cyanidin 3-rutinoside content

where: b = Hunterlab coordinate for yellow-blue

LCM = ln (cyanidin 3-rutinoside /acacetin 6-C-glucosylglycoside)

LCN = ln (cyanidin 3-rutinoside /acacetin C-diglycoside)

pH = pH of spathe sap

Cy3R = cyanidin 3-rutinoside content

ln = natural log.

Tests of the partial regression coefficients of the model showed that the order of significance of the four factors was 1) the natural log value of the ratio of cyanidin 3-rutinoside to acacetin C-diglycoside, 2) the pH of the spathes, 3) the amount of cyanidin 3-rutinoside and 4) the natural log value of the ratio of cyanidin 3-rutinoside to acacetin 6-C-glucosylglycoside (Table 4). The most important factor, the ratio between cyanidin 3-rutinoside and acacetin C-diglycoside varied directly with blueness. Thus, an increase in blueness (a decrease in Hunterlab's b value) was associated with a decrease in the copigment acacetin C-diglycoside, causing an increase in this ratio. The second most important factor, pH, also varied directly with blueness. Hence, a higher pH was associated with a more blue spathe. Of lesser

significance was the concentration of cyanidin 3-rutinoside. A lower concentration of cyanin was associated with a more purple spathe. The least significant factor was the ratio of cyanidin 3-rutinoside to acacetin 6-C-glucosylglycoside. A decrease in this ratio was associated with a more purple spathe. It should be noted that the distribution of the b values did not differ among segregating crosses.

When only the purplish spathes (RHSCC value was higher than 56) in the same populations were analyzed, the spathe pH was the only significant factor affecting the bluish hue (Table A4 and A5). Thus, an increase in the spathe pH was associated with blueness in purplish spathes.

The four factors in the best model from the segregating population were tested on the larger population consisting of the four species, 6 segregating crosses and 2 F_1 crosses (Table 2). The factors showed approximately the same order of significance as in the original population (Table A6). Moreover, the coefficient of determination in the larger population was improved from 0.38 to 0.58. This difference might be due to higher heterogeneity of Hunterlab's b value in

segregating crosses than in the species or the F_1 hybrids.

The r^2 values of all regression models in this study were low, suggesting that other factors also influenced the spathe colors and/or the quantities of factors from biochemical analyses of the spathe were not representative of the concentration of the chemicals in the pigmented cells.

In A. andraeanum, anthocyanin-containing cells are restricted to the hypodermal layers (Higaki, 1984). However, anatomical studies on other species and hybrids showed differences in the location of anthocyanin containing cells (Table 5). A. amnicola was found to have anthocyanins only in both epidermal layers. The pigment-containing cells exhibited a very uniform color within the layer where they occured. Anthocyanins in A. antioquiense and A. formosum, on the other hand, developed on only one side of the spathe. Pigmented cells were scattered in both the adaxial epidermis and adaxial hypodermis in A. antioquiense. Pigmented cells in A. formosum were dispersed in both the abaxial mesophyll and abaxial hypodermis. The cells in the two species displayed different color intensities within the

Table 5. Histological distribution of anthocyanin-containing cells in anthurium spathes.

Species	Adaxial			Abaxial		
	Epidermis	Hypodermis	Mesophyll	Epidermis	Hypodermis	Mesophyll
A. amnicola	+++++	-	-	+++++	-	-
A. andraeanum	-	+++++	-	-	+++++	-
A. antioquiense	+	+	-	-	-	-
A. bicollectivum	-	+++	+++	-	-	-
A. cerrobaulense	+++	+	+++	-	+	+++
A. coriaceum	-	+	++++	-	+	++++
A. formosum	-	-	-	-	+	+
A. friedrichsthalii	-	-	+	-	-	+
A. gladiifolium	-	-	++++	-	-	+
A. jefense	-	+	+++	-	+++	+++
A. kamemotoanum	-	+++	+++	-	-	-
A. lentii	+++	-	-	-	-	-
A. mancuniense	-	+++	+++	-	+	+++
A. plowmanii	+++++	-	+	-	+	+
A. ravenii	-	-	++++	-	-	+++++
A. superbum	+++++	++++	-	+++++	-	-
A. watermaliense	+++++	-	-	+++++	-	-
A. wendlingerii	+++++	++++	+++	+++++	++++	+++

- absent; + very few; ++ few; +++ moderate; ++++ profuse; +++++ all

tissues. The variation in the number of anthocyanin-containing cells and the difference in pigment concentration among cells may affect the accuracy of analytic data of pigments leading to the low coefficient of determination in the regression models.

The anthocyanin content has been found to be one of the important factors in color expression in anthuriums. It influences color expression both by itself and by its interactions with other factors. This finding agrees with that of Marutani (1984) that cyanidin 3-rutinoside content is positively correlated with Hunterlab's b value in anthurium spathes. The bluing effect of a decrease in cyanidin 3-rutinoside content may be due to the transmission of more of the blue spectrum after dilution (Biran, 1974b), or to an effect of the pigment-copigment ratio.

4.1.2 Factors affecting color expression in vitro

4.1.2.1 Copigmentation

Adding acacetin C-diglycoside, acacetin 6-C-glucosylglycoside or flavonol C-monoglycoside to the red cyanidin 3-rutinoside solution generally increased light absorption (color intensity) as well as shifted the absorption maxima toward red color (Table 6).

Table 6. The effects of copigmentation (at 1:1 ratio) on the spectral maxima and the absorption of cyanidin 3-rutinoside (3.17×10^{-3} M) in blue-red range at equilibrium.

Copigments	Spectral maxima (nm) pH 3.40/ pH 5.66 (shift)	Absorption (OD) pH 3.40/ pH 5.66 (change)
None	508.0/ 525.5 (0.000/ 0.000)	1.224/ 0.196 (0.000/ 0.000)
Acacetin C-diglycoside	525.0/ 543.5 (17.00/ 18.00)	1.991/ 0.749 (0.767/ 0.553)
Acacetin 6-C-glucosylglycoside	508.0/ 528.0 (0.000/ 2.500)	1.449/ 0.268 (0.225/ 0.072)
Flavonol C-monoglycoside	521.5/ 534.0 (13.50/ 8.500)	0.900/ 0.708 (-0.324/ 0.512)
Acacetin C-diglycoside and acacetin 6-C-glucosylglycoside	527.0/ 545.0 (19.00/ 19.50)	2.142/ 0.902 (0.918/ 0.706)
Acacetin C-diglycoside and flavonol C-monoglycoside	531.0/ 538.0 (23.00/ 12.50)	1.422/ 1.070 (0.198/ 0.874)
Acacetin C-diglycoside, acacetin 6-C-glucosylglycoside and flavonol C-monoglycoside	532.0/ 542.5 (24.00/ 17.00)	1.400/ 0.917 (0.176/ 0.721)

Table 7. Statistical summary of the data of factors involving anthurium spathe colors.

Variable	No. observation	Mean	Std Dev	Maximum	Minimum
RHSCC	220	-	-	84C	52A
Hunterlab's b	220	3.268	6.234	17.88	-10.18
pH	220	5.661	0.241	6.25	5.02
Cyanidin 3-rutinoside*	220	6834	9171	65766	63
Peonidin 3-rutinoside*	220	324	603	4046	0
Total anthocyanins*	220	7414	9921	65766	63
Acacetin C-diglycoside*	220	8143.077	12841.000	72100.00	0.00
Acacetin 6-C-glucosyl-glycoside*	206	8132.127	8025.200	45296.00	0.00
Flavonol C-monoglycoside*	220	14431.250	21932.000	100306.00	0.00
Total organic acid*	42	74126	28010	120928.00	11586.00
Iron (ppm)	39	33.615	19.484	67.00	5.00
Magnesium (ppm)	39	3610.000	992.000	5950.00	2050.00

* Data of these variables were measured in integrator area unit/ fresh weight (volt/gram).

At pH 5.66 which was the average pH of the spathe sap (Table 7), the flavone acacetin C-diglycoside from A. andraeanum produced the most bathochromic shift (+18 nm) for any single copigment, whereas the acacetin 6-C-glucosylglycoside from A. amnicola and A. antioquiense showed the least shift (+2.5 nm). Acacetin C-diglycoside exhibited the highest increase of absorbance at the absorption maxima (0.553), while acacetin 6-C-glucosylglycoside exhibited the lowest (0.072). Thus, acacetin C-diglycoside was the most effective copigment for blue color enhancement in this study while acacetin 6-C-glucosylglycoside was the least effective.

Flavonol C-monoglycoside, the major flavonol found in both A. antioquiense and A. formosum, showed antagonistic effects on the bathochromic shift induced by acacetin C-diglycoside and a mixture of the two flavone C-glycosides, acacetin C-diglycoside and acacetin 6-C-glucosylglycoside, at pH 5.66. These observations suggest that copigmentation of cyanidin 3-rutinoside with flavone C-glycoside and flavonol C-glycoside was a competitive process.

Copigmentation is an important phenomenon in color expression in flowers since the same anthocyanin can

display different colors. This phenomenon does not only produce the bluing effect but also stabilizes and amplifies the color of anthocyanins (Asen, 1976, Osawa, 1982). An earlier *in vitro* study in anthuriums suggested that a flavonol C-monoglycoside was a copigment in purple *A. formosum* hybrids (Marutani, 1984).

From the regression analysis, the effect of flavonol C-monoglycoside content was found to be insignificant. *In vitro* experiments nevertheless confirmed Marutani's result (1984) that the flavonol was capable of copigmentation. The *in vitro* data suggest the following: the flavonol may be unavailable to form a complex with cyanidin 3-rutinoside in the natural environment since they may belong to different cell types or cell compartments; the analytical data may not reflect the natural concentration of the copigment; or the flavonol may actually be an artifact of an unstable compound.

The ratio of cyanidin 3-rutinoside to acacetin C-diglycoside was found to be the most important variable in the regression model. This C-glycosyl flavone exerts a negative effect on Hunterlab's b value, suggesting an antagonism among flavonoids in the formation of the 'blue' pigment complex. However, *in vitro* experiments showed that acacetin C-diglycoside induced the greatest

bathochromic shift among major flavonoids from the four Anthurium species. Differences between the regression analysis and experimental results may be accounted for by an antagonism due to some unknown factors that might strongly associate with the flavonoid copigment. Another possibility is that a flavonoid which expresses limited solubility in vitro may behave differently in its natural environment.

Flavone 6-C-glycoside was reported to be one of the most powerful class of copigments (Asen et al., 1972). In this study, a 6-C-glycosyl flavone, acacetin 6-C-glucosylglycoside, from A. amnicola was important in the regression models. However, this flavonoid exhibits only a slight bathochromic shift in vitro.

All copigments involved in this study showed a disparity between the results from the regression analysis and those of the in vitro experiments. An anatomical study showed that the purplish hue was consistently greater in the colored epidermis than in the hypodermis and the mesophyll (Figure 1). Thus, it is possible that the histological distribution of the anthocyanins and the flavonoids in different tissue layers of the spathes may be a significant factor in color expression in anthurium.

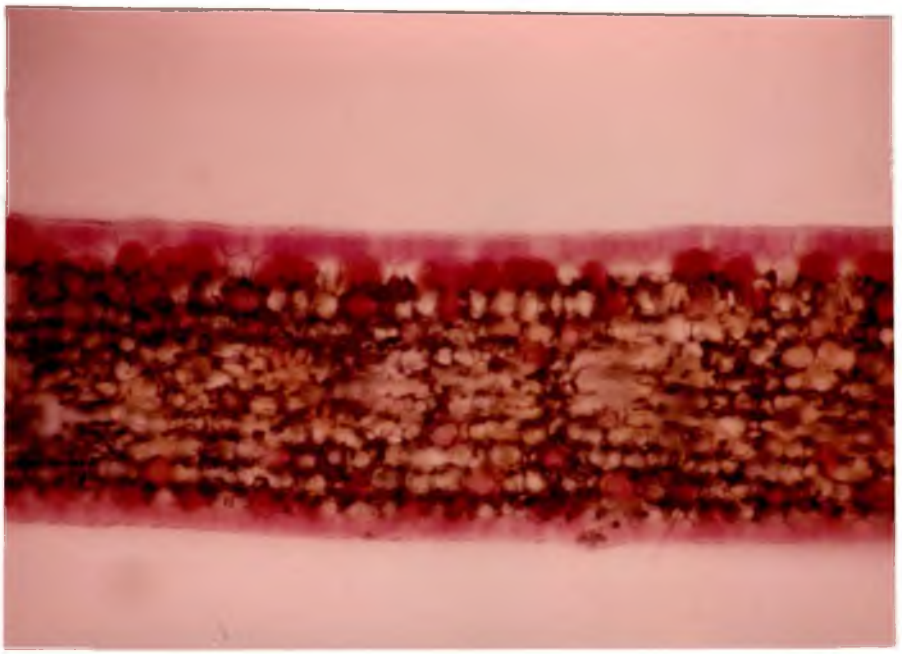

Figure 1. Cross section of a purplish spathe (plant number 763-7) showing the heterogeneous nature of pigmented cells.

4.1.2.2 pH

The effect of pH on light absorption seems to be related to the stability of anthocyanins. With an increase of pH, a bathochromic shift would take place while the absorption would decrease (Asen et al., 1971a; Jurd and Asen, 1966; de Loose, 1978; Yazaki, 1976). Earlier work on anthurium suggested that pH might be one of the contributing factors in color variation (Marutani, 1984).

At equilibrium (18 hours at 12°C), the absorbance of cyanidin 3-rutinoside solution increased by 2% at pH 3.40, whereas the absorbance decreased 88.86% at pH 5.66 (Table 8). pH also affected the spectral maxima of cyanidin 3-rutinoside. Reduction of pH exhibited hypsochromic shift of 17.5 nm. In general, cyanidin 3-rutinoside and its mixtures were bluer and lighter at higher pHs (Table 6).

Copigmentation was also influenced by pH. The effects of copigments on light absorption of cyanidin 3-rutinoside appeared to be pH-specific. Acacetin 6-C-glucosylglycoside induced a bathochromic shift (2.5 nm) at pH 5.66 but not at pH 3.40. In contrast, the bathochromic shift of flavonol C-monoglycoside was higher at pH 3.40 than at pH 5.66.

Table 8. The effects of copigmentation (at 1:1 ratio) on stability of cyanidin 3-rutinoside (3.17 x 10^{-3} M).

Copigments	Absorption (OD) at spectral maxima	
	pH 3.40 0/18 hrs	pH 5.66 0/18 hrs
None	1.199/ 1.224 (+ 2.08%)*	1.768/ 0.196 (- 88.91%)
Acacetin C-diglycoside	2.002/ 1.991 (- 0.05%)	2.318/ 0.749 (- 67.69%)
Acacetin 6-C-glucosylglycoside	1.454/ 1.449 (- 0.03%)	2.066/ 0.268 (- 87.03%)
Flavonol C-monoglycoside	0.904/ 0.900 (- 0.04%)	2.322/ 0.708 (- 69.51%)
Acacetin C-diglycoside and acacetin 6-C-glucosylglycoside	2.104/ 2.142 (+ 1.81%)	2.592/ 0.902 (- 65.20%)
Acacetin C-diglycoside and flavonol C-monoglycoside	1.404/ 1.422 (+ 1.28%)	2.279/ 1.070 (- 53.05%)
Acacetin C-diglycoside, acacetin 6-C-glucosylglycoside and flavonol C-monoglycoside	1.350/ 1.400 (+ 3.70%)	1.908/ 0.917 (- 51.94%)

* Numbers in parenthesis indicates the change of OD at spectral maxima.

In vitro data suggested that pH interacted with both cyanidin 3-rutinoside and types of copigmentation. Although regression analyses could not account for the interaction effects of pH on either cyanidin 3-rutinoside quantity or types of copigmentation, the statistical method showed the effects of pH to be the second most significant.

4.2 Genetics of Lavender-Purple Spathe

4.2.1 Cross compatibility

Interspecific hybridization between *A. amnicola* and other species is necessary for the improvement of anthurium cultivars with large lavender-purple spathes. Therefore, a study of cross compatibility should be useful in the breeding program. Results of half-diallel crosses among *A. amnicola*, *A. andraeanum*, *A. antioquiense* and *A. formosum* are shown in Table 9. Every hybrid combination produced a reasonably high number of viable seeds, indicating a close relationship among the four species. The number of seedlings per fruit of the intragroup hybrid of group VI (*A. andraeanum* x *A. formosum*) was 41.8% lower than that of the hybrid of group I (*A. amnicola* x *A. antioquiense*)(Table 10). Based on the limited data from the half-diallel crosses,

Table 9. Cross compatibility among *A. amnicola*, *A. andraeanum*, *A. antioquiense* and *A. formosum*.

Cross number	Parents	Number of fruits	seeds	seedlings	seedlings/fruit
725	*A. antioquiense* x *A. formosum*	106	170	146	1.38
729	*A. antioquiense* x *A. amnicola*	107	184	179	1.67
752	*A. andraeanum* 'Marian Seefurth' x *A. formosum*	110	138	107	0.97
756	*A. andraeanum* 'Marian Seefurth' x *A. amnicola*	59	71	52	0.88
768	*A. antioquiense* x *A. andraeanum* 'Marian Seefurth'	93	103	94	1.01
789	*A. amnicola* x *A. formosum*	39	54	43	1.10

Table 10. Crossability of four anthurium species in a half-diallel crossing block.

Parent	Number of seedling/ fruit		
	intragroup	intergroup	composite
Group I			
A. amnicola	1.67	0.97	1.33
A. antioquiense	1.67	1.21	1.37
Group VI			
A. andraeanum 'Marian Seefurth'	0.97	0.96	0.97
A. formosum	0.97	1.30	1.16

A. antioquiense appeared to have the best crossability, while A. andraeanum 'Marian Seefurth' seemed to have the worst crossability. A. formosum showed the highest intergroup crossability among the four species in the half-diallel.

4.2.2 Meiotic analysis and pollen stainability of the interspecific hybrids

Chromosome pairing at metaphase I of all hybrids among the four species was regular (Table 11, Fig. 2). The formation of 15 bivalents at metaphase I indicated good chromosome homology among the four species although they belong to two groups or sections. A. antioquiense (A 490A) had two B chromosomes, and its offspring showed the transmission of the B chromosomes (Table 11).

At late metaphase I in A. amnicola and some hybrids, one or two pairs of chromosomes moved away from the equatorial plate and showed spindle abnormality. The pairs of precocious chromosomes frequently migrated to the same pole (Fig. 3). As a result, tetrads from these pollen mother cells would be aneuploid. Aneuploid microspores often suffer physiological disorders and die. This nondisjunction phenomenon has been observed in various plant families and is suggested to be the result

Table 11. Mean meiotic configuration at metaphase I and pollen stainability in aceto-carmine of anthurium species and hybrids.

Accession or cross numbers	Species or cross	Mean configuration (25 cell/plant)	% Pollen stainability (1000 cell/spadix)
A 417	A. amnicola	15II (1)*	84.7 (1)*
H 33	A. andraeanum 'Marian Seefurth'	15II (1)	48.6 (1)
A 490A	A. antioquiense	15II + 2B (1)	88.5 (1)
A 290	A. formosum	15II (1)	88.5 (1)
725	A. antioquiense x A. formosum	15II + 1.2B (5)	36.3 (7)
729	A. antioquiense x A. amnicola	15II + 1.2B (5)	9.5 (7)
752	'Marian Seefurth' x A. formosum	15II (5)	24.4 (7)
756	'Marian Seefurth' x A. amnicola	15II (5)	11.1 (7)
768	A. antioquiense x 'Marian Seefurth'	15II + 1.6B (5)	50.4 (7)
789	A. amnicola x A. formosum	15II (5)	3.7 (7)

* Number in parenthesis indicates number of plants examined.

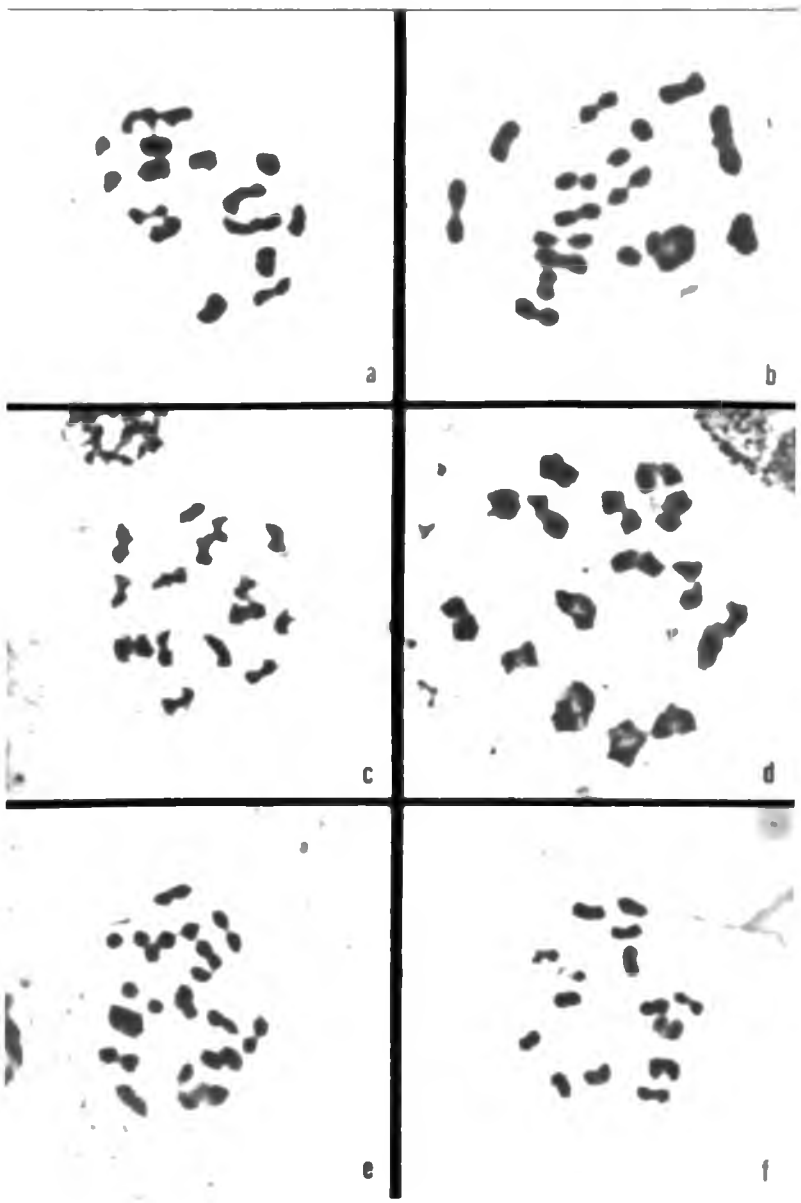

Figure 2. Meiotic configurations of six interspecific hybrids:
a) 725-11 (A. antioquiense x A. formosum) (15II+1B)
b) 729-17 (A. antioquiense x A. amnicola) (15II+1B)
c) 752-1 ('Marian Seefurth' x A. formosum) (15II)
d) 756-20 ('Marian Seefurth' x A. amnicola) (15II)
e) 768-24 (A. antioquiense x 'Marian Seefurth) (15II +1B)
f) 789-40 (A. amnicola x A. formosum) (15II).

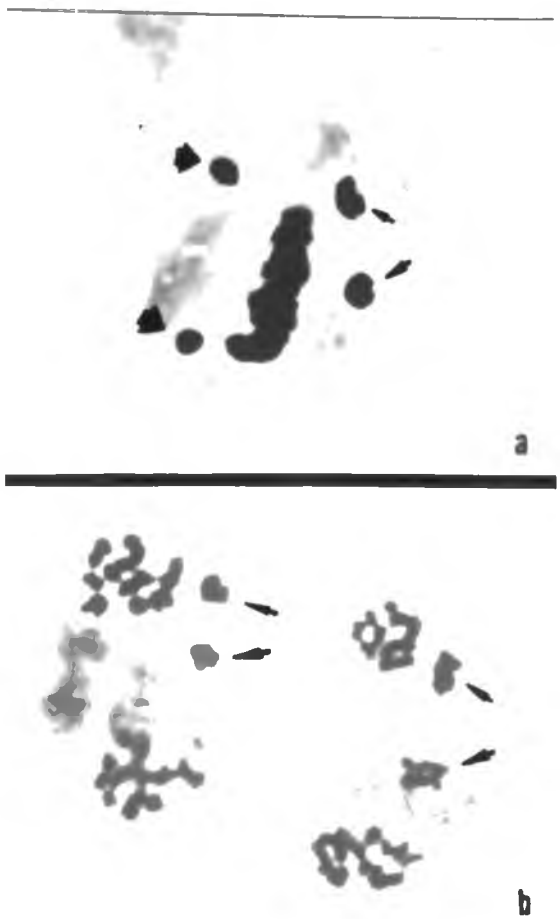

Figure 3. Abnormal chromosome movement in:
a) Late metaphase I (725-39)(A. antioquiense x A. formosum)
b) Metaphase II (A. amnicola).

(narrow arrow indicates irregular autosomes and broad arrow indicates irregular B chromosomes)

of gene action (Arisumi, 1987; Swanson and Nelson, 1937) or an environmental effect (Swanson, 1942).

Analysis of tetrad formation was excluded from this study since earlier data (Marutani, 1984) indicated that tetrad formation had very low correlation with pollen stainability (r=0.0173). Tetrad formation could be normal even if there were irregular chromosome movement as mentioned above. Thus the analysis of tetrad formation is not a good index of meiotic abnormality in anthuriums.

Pollen stainability of the four species and their hybrids was examined. A. andraeanum 'Marian Seefurth' produced the lowest percentage of viable pollen grains among the parents, possibly as a result of the hybrid origin of cultivated anthuriums (Kaneko and Kamemoto, 1978). Unlike A. andraeanum 'Kaumana' and 'Uniwai', 'Marian Seefurth' did not show irregular meiotic behavior at metaphase I. Marutani (1984) hypothesized that meiotic irregularity might occur late in metaphase I despite regular pairing at prometaphase I.

Among interspecific hybrids, A. amnicola progenies exhibited the lowest percentage of viable pollen grains while A. antioquiense offspring showed the highest (Table 12). The hybrid between A. antioquiense and 'Marian

Table 12. Pollen viability resulting from half-diallel crosses of four anthurium species.

Parental species	% Pollen stainability		
	intragroup	intergroup	composite
Group I			
A. amnicola	9.50	7.40	8.10
A. antioquiense	9.50	43.35	32.07
Group VI			
A. andraeanum 'Marian Seefurth'	24.40	30.75	28.63
A. formosum	24.40	20.00	21.47

Seefurth' was the most fertile. Although, its pollen stainability was comparable to that of 'Marian Seefurth', the hybrid produced pollen year-round unlike 'Marian Seefurth' which produced pollen mostly in winter months.

Pollen stainability studies have their limitation in some interspecific hybrids, particularly in A. amnicola hybrids. Anther emergence of these hybrids was observed to be strongly affected by environmental factors such as humidity and temperature. Such factors may also affect pollen viability.

Since meiotic pairing in interspecific hybrids was regular, the reduction of pollen viability does not seem to be a direct result of any abnormality in meiotic pairing. However, the spindle abnormalities observed in some species and hybrids, and genic male sterility may be important reproductive barriers between species of this genus.

4.2.3 Inheritance studies

4.2.3.1 Qualitative inheritance

Since the pigmented cells are unequally distributed between abaxial and adaxial sides of anthurium spathes (Table 5), only the adaxial color was considered in this study.

The lavender-purple spathe color appears to be a recessive trait because most first generation hybrids between the lavender A. amnicola and other species have never produced purplish spathes. Only A. amnicola x A. antioquiense and A. amnicola x A. formosum produced lavender-purple spathes (Table 13). A backcross to A. amnicola [cross 634, (A. lindenianum x A. amnicola) x A. amnicola] produced a 1:1 ratio of red and purple. Hybrids between the purplish spathe species, A. amnicola or A. antioquiense, and species or plants with other spathe colors (except A. formosum) produced only pink-red spathes (Table 13), suggesting that the gene controlling lavender-purple color is recessively epistatic to genes for other colors.

All species involved in this study were assumed to be homozygous for the locus/loci controlling the lavender-purple color, since spathe colors have been found to be consistent for color groups within species (Croat, 1983; Croat, 1986). The genes M and O have been reported to control the production of the anthocyanins, cyanidin 3-rutinoside and pelargonidin 3-rutinoside, respectively (Iwata, 1980). Among the four parents of half-diallel crosses, A. andraeanum was the only species that did not produce hybrids with purplish spathes. It

Table 13. First generation hybrids between A. amnicola and other anthurium species of purple (P), pink-red (R), orange (O) and white (W) spathes.

Cross numbers	Parents	Spathe colors of progenies
531, 558	'Calypso' (R) x A. amnicola (P)	Pink-Red
552, 559	A. lindenianum (W) x A. amnicola (P)	Pink-Red
568, 572	A. amnicola (P) x A. formosum (W)	Purple
569, 575	A. amnicola (P) x A. lindenianum (W)	Pink-Red
576	A. antrophyoides (W) x A. amnicola (P)	Pink
717, 718	A. andraeanum (O) x A. amnicola (P)	Pink-Red
729	A. antioquiense (P) x A. amnicola (P)	Purple
755	A. andraeanum 'Uniwai' (W) x A. amnicola (P)	Pink-Red
756	A. andraeanum 'Marian Seefurth' (R) x A. amnicola (P)	Pink-Red

was also the only species that produced acacetin C-diglycoside, the negative regression factor for the blueness of the spathe. Therefore, it is likely that A. andraeanum is a homozygote for a dominant allele controlling the presence of the negative factor for copigmentation. Locus P is postulated to be involved in the copigmentation-antagonist production. The dominant allele P was hypothesized to control the high production of the antagonistic factor, while the recessive allele p controls the absence or low production. Thus, the genotype pp allows formation of the anthocyanin-copigment complex leading to a color change of the anthocyanin. The genotype O pp will yield a purple spathe if allele M is present, while it will yield a red spathe if the locus M is homozygous recessive.

In Table 14, fifteen crosses which produced purple-red segregating offspring are placed into six groups. Offspring of purple x red parents segregated into a 1:1 ratio of purple and red. This suggests that the red parents are heterozygous at the P locus and that the purple parents are homozygous recessive at the P locus. Moreover, the two parents must have been homozygous dominant at the M and O loci. Therefore, the genotype

Table 14. Segregating populations from 15 crosses involving purple, red and white spathes in Anthurium.

Color of parents (cross numbers)	Phenotypes of offspring Purple	Red	White	Chi^2	P
Purple x Red (573, 587, 634 and 733)	59 (1:	60 1:	0 0)*	0.008	<0.90
Purple x White (751)	9 (1:	0 0:	6 1)	0.600	<0.10
Red x White (739)	3 (1:	8 1:	0 0)	2.273	<0.10
Red x White (692, 696, 697, 753 and 762)	36 (1:	19 1:	49 2)	5.904	<0.05
Red x Red (741 and 758)	19 (3:	28 3:	19 2)	2.505	<0.10
Red x Red (747 and 750)	10 (3:	15 9:	7 4)	3.291	<0.10

* Number in parenthesis indicates expected ratio.

MMOOPp was assigned to the red parents and MMOOpp to the purple parents.

Crosses between purple and white parents yielded purple and white offspring in a 1:1 ratio, suggesting a one-gene difference between purple and white at the O locus. The genotypes of the two parents at the locus M must have been homozygous dominant, while at the locus P must have been homozygous recessive. The genotype of the purple-spathe parent was designated as MMOopp, while that of the white-spathe parent as MMoopp.

There are two types of red and white crosses. The first type produced purple and red progenies in a 1:1 ratio. In this case, the red parent must have been heterozygous at the P locus with a MMOOPp genotype. The white parent must have been MMoopp. The second type gave purple, red and white in a 1:1:2 ratio, indicating segregation at 2 loci. In this case, the genotype of the red parent was MMOoPp and the white parent again was MMoopp.

There were also two types of red x red crosses. One gave a ratio of 3:3:2 red: purple: white. Both parents were heterozygous at the P locus, but only one at the O locus, ie. MMOoPp and MMOOPp. The second type gave a

9:3:4 recessive epistatic ratio which is expected from a cross of two parents with the MMOoPp genotype.

The following genotypes are proposed for the parental species: MMOOpp for A. amnicola (lavender), MmOoPP for A. andraeanum 'Marian Seefurth' (pink), MMOOpp for A. antioquiense (pale pinkish lavender), MMoopp for A. formosum (cream), mmooPP for A. lindenianum (white) and MMOOPP for A. kamemotoanum (red). An accession of A. formosum (A 507) which produced an unusual reddish purple spathe would probably have the genotype MMOopp.

In summary, the O locus is recessively epistatic to the loci M and P. If the O locus is recessive, the color is white. When the M and O locus are dominant, then the spathe is purple if the P locus is recessive, red if it is dominant. When both loci O and P are dominant, the spathe is orange if the locus M is recessive. There is no case in this study where the M and P loci are recessive; however, the red color is speculated for this group of genotypes since the product of the genotype mmO_, pelargonin, is unable to form a purplish copigmentation complex (Asen, 1976).

4.2.3.2 Quantitative inheritance

When the spathe color was classified discretely, the Chi-square test for the phenotypic ratio was statistically accepted. However, the low level of acceptability may be due to the misclassification of the color of some individuals since the purple-red color is somewhat continuous. The Hunterlab's b value was then treated as a quantitative trait because it was a continuous value and was also affected by at least four biochemical factors. Half-diallel hybrids from A. amnicola, A. andraeanum 'Marian Seefurth', A. antioquiense and A. formosum were used to analyze biochemical data of all factors influencing Hunterlab's b value of the spathes. The general combining abilities (GCA) of each species were then computed.

From Table 15, A. amnicola was the best parent in producing purplish spathe hybrids. The species had high GCA for b value and was also shown to provide its offspring with high pH and high acacetin 6-C-glucosyl-glycoside, the important factors determining purplish spathe.

'Marian Seefurth' appeared to be the poorest parent in creating lavender-purple anthuriums since its GCA for

Table 15. General combining ability for Hunterlab's b value and the four biochemical factors influencing b values, of the four Anthurium species.

Species	b	Acacetin C-diglycoside	Acacetin 6-C-glucosylglycoside	Cyanidin 3-rutinoside	pH
A. amnicola	- 5.018	1230.0	2691.0	- 4818.5	0.13
A. andraeanum 'Marian Seefurth'	5.788	14808.5	- 1775.5	6189.5	- 0.19
A. antioquiense	0.008	- 6646.5	2131.5	- 7253.5	0.14
A. formosum	- 0.778	- 9392.0	- 3047.0	5882.5	- 0.08

Hunterlab's b value was the most positive. In addition, the cultivar was good in providing its hybrids with high cyanidin 3-rutinoside, acacetin C-diglycoside production and pH reduction, which were important in reddish color.

A. formosum was the best parent in reducing acacetin C-diglycoside. However, this species produced progenies with high cyanidin 3-rutinoside content, low acacetin 6-C-glucosylglycoside production and low pH. It was noted that a backcross hybrid (cross 762) of A. formosum without A. amnicola or A. antioquiense in the pedigree produced some offspring with purple spathes.

The interesting species for further hybridization programs is A. antioquiense. Despite its low GCA for b value, it increased the pH and acacetin 6-C-glucosyl-glycoside, and decreased acacetin C-diglycoside and cyanidin 3-rutinoside in hybrid populations.

4.3 General Discussion

Based on biochemical data, a regression model was constructed to predict the blueness of anthurium spathes in segregating populations. The in vitro experiment yielded a disparity suggesting that the cytological environment of pigmented cells seems to play an important role. Biochemical data then should be used cautiously

for the purpose of predicting only, and not for the explanation of the pigment behavior for color expression in anthuriums. Furthermore, the model does not account for white spathes in the population.

Further investigation should be done to obtain a regression model with a higher coefficient of determination. Analytical methods at the cellular level, such as measurement of pH by microspectrophotometry, should be employed. Other metal ions or types and forms of organic acid should also be examined. Extensive collection of both cyanidin 3-rutinoside and its cofactors is necessary for performing *in vitro* experiments.

Crossability and hybrid analysis indicated close relationships among *A. amnicola* and *A. antioquiense* of section *Porphyrochitonium*, and *A. andraeanum* and *A. formosum* of section *Calomystrium*. Meiotic analysis at metaphase I showed good pairing indicating good genome homology between the two sections. Irregularity of chromosome movement and pollen sterility in the hybrids suggest the presence of species barriers other than chromosome structure.

A genetic system controlling lavender-purple colors on the adaxial side of anthurium spathes is proposed

utilizing the results of regression analysis and phenotypic data of segregating and half-diallel population. An allele P is hypothesized to control high production of a copigmentation competitor found in A. andraeanum. This allele is recessively epistatic to the loci controlling cyanidin 3-rutinoside production. Another important factor is cyanidin 3-rutinoside which is controlled by an epistatic system of alleles M and O. High pH is suggested to be a secondary factor whose role is to enhance the blueness.

In a breeding program for lavender-purple anthuriums, the use of several species are necessary to incorporate the good features of each species. The general combining ability study indicated that A. antioquiense had a potential to be a good parental species in producing lavender-purple hybrids. This species generally produced hybrids with low production of acacetin C-diglycoside and cyanidin 3-rutinoside, and high production of acacetin 6-C-glucosylglycoside and high pH.

Although A. andraeanum 'Marian Seefurth' seems to be unfavorable for increasing the blueness in hybrids, its spathe shape, size and vase-life are desirable. Hybrids of A. andraeanum should be sibbed and/or half-sibbed to

create recombinant populations for purple spathes with other desirable traits.

A. amnicola has the highest GCA for purple spathes. However, a minimal use of the species is recommended because its hybrids rarely produce viable pollen.

Despite the high GCA for b value, A. formosum is not a promising parent because of some undesirable characteristics, such as short peduncles hidden beneath the foliage. However, this species may be useful for creating large tulip-type lavender-purple anthuriums.

Improvement of lavender-purple anthuriums apparently needs an appropriate hybrid combination involving the four species studied. An anthurium with a bluer and larger spathe than A. amnicola could possibly be accomplished by a hybridization strategy that will utilize biochemical and genetic information to select the 'right' parents. The ideal hybrids should have low content of acacetin C-diglycoside, high pH, low content of cyanidin 3-rutinoside and high content of acacetin 6-C-glucosylglycoside.

APPENDICES

Table A1. ANOVA for relationships between Hunterlab's b values and selected factors in cross 587.

Analysis of Variance

Dependent variable: Hunterlab's b

Source	Df	Sum of square	Mean square	F Value	Pr>F
Model	4	511.121	127.780	3.25	0.0256
Error	29	1140.830	39.339		
Total	33	1651.951			

Mean	S.D.	C.V.	R-square
0.934	6.272	671.233	0.30941

Source*	Df	Type I SS	Mean square	F Value	Pr>F
pH	1	404.654	404.654	10.29	0.0033
LAC	1	76.367	76.367	1.94	0.1741
O-Acid	1	27.976	27.976	0.71	0.4060
Fe+Mg	1	2.124	2.124	0.05	0.8179

Source*	Df	Type III SS	Mean square	F Value	Pr>F
pH	1	374.040	374.040	9.51	0.0045
LAC	1	94.324	94.324	2.40	0.1324
O-Acid	1	30.001	30.001	0.76	0.3897
Fe+Mg	1	2.124	2.124	0.05	0.8179

* pH = pH of spathe sap
LAC = ln [(cyanidin 3-rutinoside + peonidin 3-rutinoside)/(acacetin C-diglycoside + acacetin 6-C-glucosylglycoside + flavonol C-monoglycoside)]
O-Acid = total organic acids content
Fe+Mg = Iron and magnesium content

Table A2. ANOVA for relationships between Hunterlab's b values and selected factors in segregating populations of crosses 573, 587, 634, 692, 693 and 763.

Analysis of Variance

Dependent variable: Hunterlab's b

Source	Df	Sum of square	Mean square	F Value	Pr>F
Model	9	1453.117	161.457	5.32	0.0001
Error	68	2032.207	30.331		
Total	76	3485.324			

b Mean	S.D.	C.V.	R-square
1.205	5.507	456.972	0.41693

Source*	Df	Type I SS	Mean square	F Value	Pr>F
Cross	5	163.725	32.745	1.08	0.3797
LCM	1	403.054	403.054	13.29	0.0005
LCN	1	309.287	309.287	10.20	0.0021
pH	1	421.743	421.743	13.90	0.0004
Cy3R	1	155.307	155.307	5.12	0.0269

Source*	Df	Type III SS	Mean square	F Value	Pr>F
Cross	5	124.200	24.840	0.82	0.5405
LCM	1	175.779	175.779	5.80	0.0188
LCN	1	448.008	448.008	14.77	0.0003
pH	1	385.858	385.858	12.72	0.0007
Cy3R	1	155.307	155.307	5.12	0.0269

* b = Hunterlab coordinate for yellow-blue
LCM = ln (cyanidin 3-rutinoside /acacetin 6-C-glucosylglycoside)
LCN = ln (cyanidin 3-rutinoside /acacetin C-diglycoside)
pH = pH of spathe sap
Cy3R = cyanidin 3-rutinoside content

Table A3. ANOVA for relationships between Hunterlab's b values and selected factors in segregating populations of crosses 573, 587, 634, 692, 693 and 763.

Analysis of Variance

Dependent variable: Hunterlab's b

Source	Df	Sum of square	Mean square	F Value	Pr>F
Model	4	1328.917	332.229	11.09	0.0001
Error	72	2156.407	29.950		
Total	76	3485.324			

b Mean	S.D.	C.V.	R-square
1.205	5.473	454.090	0.38129

Source*	Df	Type I SS	Mean square	F Value	Pr>F
LCM	1	310.335	310.335	10.36	0.0019
LCN	1	497.309	497.309	16.60	0.0001
pH	1	298.793	298.793	9.98	0.0023
Cy3R	1	222.479	222.479	7.43	0.0081

Source*	Df	Type III SS	Mean square	F Value	Pr>F
LCM	1	177.274	177.274	5.92	0.0175
LCN	1	698.781	698.781	23.33	0.0001
pH	1	310.993	310.993	10.38	0.0019
Cy3R	1	222.479	222.479	7.43	0.0081

* b = Hunterlab coordinate for yellow-blue
LCM = ln (cyanidin 3-rutinoside /acacetin 6-C-glucosylglycoside)
LCN = ln (cyanidin 3-rutinoside /acacetin C-diglycoside)
pH = pH of spathe sap
Cy3R = cyanidin 3-rutinoside content

Table A4. ANOVA of the selected model for Hunterlab's b value in the purplish group of the six segregating crosses.

Analysis of Variance

Dependent variable: Hunterlab's b

Source	Df	Sum of square	Mean square	F Value	Pr>F
Model	4	250.623	62.656	3.59	0.0132
Error	42	733.316	17.460		
Total	46	983.939			

b Mean	S.D.	C.V.	R-square
-3.152	4.179	132.561	0.25471

Source*	Df	Type I SS	Mean square	F Value	Pr>F
LCM	1	43.499	43.499	2.49	0.1220
LCN	1	118.454	118.454	6.78	0.0127
pH	1	87.168	87.168	4.99	0.0308
Cy3R	1	1.504	1.504	0.09	0.7706

Source*	Df	Type III SS	Mean square	F Value	Pr>F
LCM	1	42.089	42.089	2.41	0.1280
LCN	1	47.349	47.349	2.71	0.1071
pH	1	88.540	88.540	5.07	0.0296
Cy3R	1	1.504	1.504	0.09	0.7706

* b = Hunterlab coordinate for yellow-blue
LCM = ln (cyanidin 3-rutinoside /acacetin 6-C-glucosylglycoside)
LCN = ln (cyanidin 3-rutinoside /acacetin C-diglycoside)
pH = pH of spathe sap
Cy3R = cyanidin 3-rutinoside content

Table A5. ANOVA of the selected model for Hunterlab's b value in the purplish group of the six segregating crosses.

Analysis of Variance

Dependent variable: Hunterlab's b value

Source	Df	Sum of square	Mean square	F Value	Pr>F
pH	1	184.997	184.997	10.74	0.0019
Error	51	878.572	17.227		
Total	52	1063.569			

b Mean	S.D.	C.V.	R-square
-3.024	4.150	137.255	0.17394

Table A6. ANOVA of the selected model for Hunterlab's b values in the general population.

Analysis of Variance

Dependent variable: b

Source	Df	Sum of square	Mean square	F Value	Pr>F
Model	12	3341.451	278.454	14.25	0.0001
Error	123	2403.133	19.538		
Total	135	5744.584			

b Mean	S.D.	C.V.	R-square
3.582	4.420	123.414	0.58167

Source*	Df	Type I SS	Mean square	F Value	Pr>F
CROSS	8	2082.562	260.320	13.32	0.0001
LCM	1	428.766	428.766	21.95	0.0001
LCN	1	234.088	234.088	11.98	0.0007
pH	1	345.288	345.288	17.67	0.0001
Cy3R	1	250.747	250.747	12.83	0.0005

Source*	Df	Type III SS	Mean square	F Value	Pr>F
CROSS	8	893.642	111.705	5.72	0.0001
LCM	1	269.070	269.070	13.77	0.0003
LCN	1	422.970	422.970	21.65	0.0001
pH	1	307.307	307.307	15.73	0.0001
Cy3R	1	250.747	250.747	12.83	0.0005

* b = Hunterlab coordinate for yellow-blue
LCM = ln (cyanidin 3-rutinoside /acacetin 6-C-glucosylglycoside)
LCN = ln (cyanidin 3-rutinoside /acacetin C-diglycoside)
pH = pH of spathe sap
Cy3R = cyanidin 3-rutinoside content

Table A7. Some biochemical data of the segregating population of crosses 573, 587, 634, 692, 693 and 763.

Variable	Color	No. observation	Mean	Std Dev	Maximum	Minimum
Hunterlab's b value	Purple	53	- 3.02	4.52	6.35	- 10.18
	Red	32	8.17	2.67	13.50	0.80
pH	Purple	53	5.66	0.24	6.25	5.20
	Red	32	5.56	0.18	5.95	5.25
Cyanidin 3-rutinoside*	Purple	53	6943	4851	18540	393
	Red	32	9759	8267	33176	231
Acacetin C-diglycoside*	Purple	53	1793	1612	9150	0
	Red	32	5885	8152	32702	0
Acacetin 6-C-glucosyl-glycoside*	Purple	53	14351	11386	45296	0
	Red	32	7614	6684	23878	376

* Data of these variables were measured in integrator area unit/ fresh weight (volt/ gram).

Table A8. Mean value of Hunterlab's b values and biochemical factors involved in color determination in some anthuriums.

Species and crosses	b*	Acacetin C-diglycoside	Acacetin 6-C-glucosylglycoside	Cyanidin 3-rutinoside	pH
A. amnicola (M)	- 6.73	-	21056.00	7094.40	5.96
A. andraeanum 'Marian Seefurth' (N)	8.54	31118.00	-	4164.60	5.05
A. antioquiense (Q)	6.32	-	8908.00	533.10	5.67
A. formosum (F)	1.62	-	-	1064.80	5.07
725 (Q x F)	6.03	-	1657.47	612.93	5.65
729 (Q x M)	- 1.29	-	9645.07	908.53	5.91
752 (N x F)	8.73	10832.80	-	25052.40	5.38
756 (N x M)	7.57	32077.60	3488.27	3355.27	5.52
768 (Q x N)	8.83	16324.40	8377.20	4525.67	5.80
789 (M x F)	- 2.76	-	7664.80	6653.07	5.90

* Hunterlab's b value

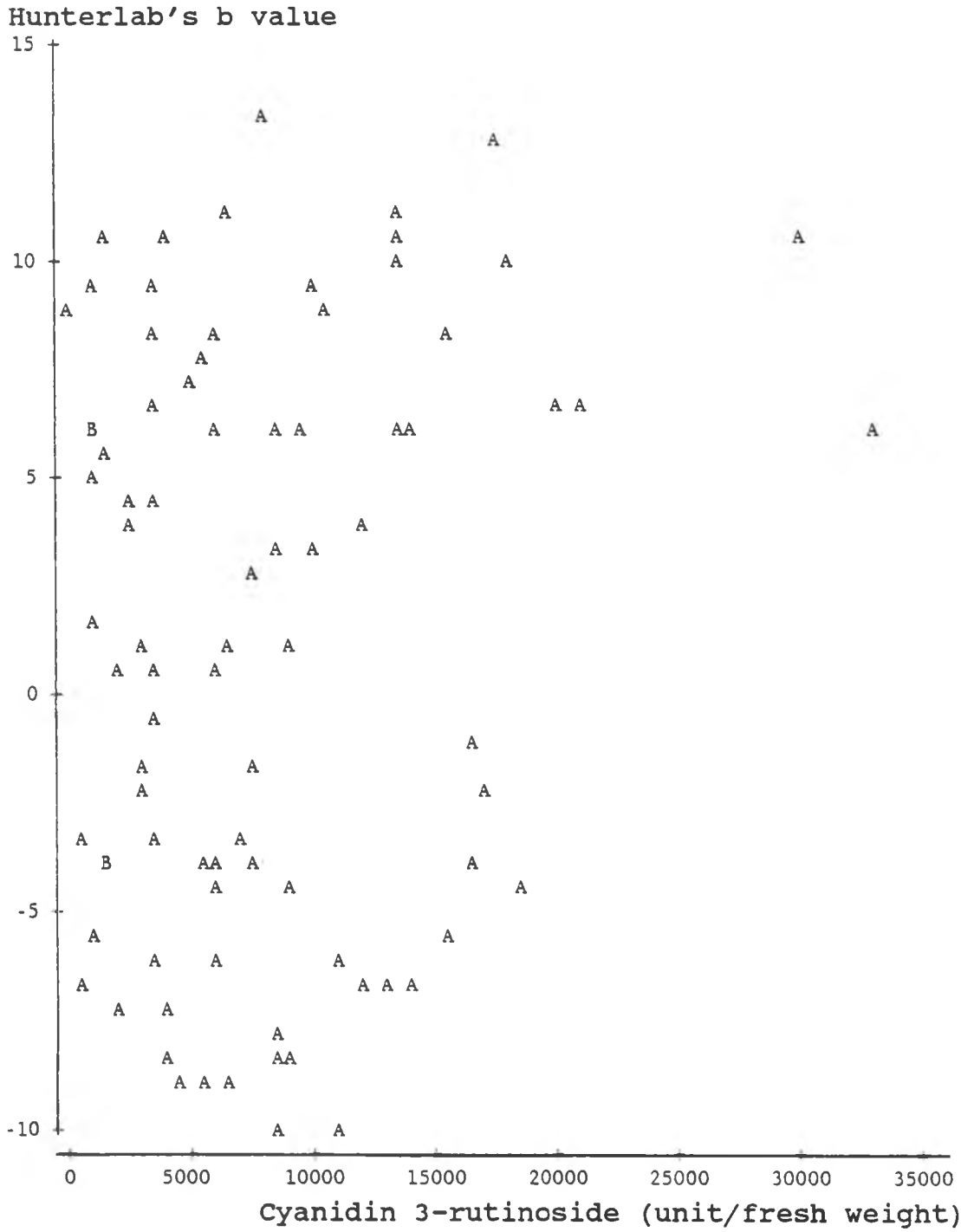

Figure A1. The distribution of cyanidin 3-rutinoside in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

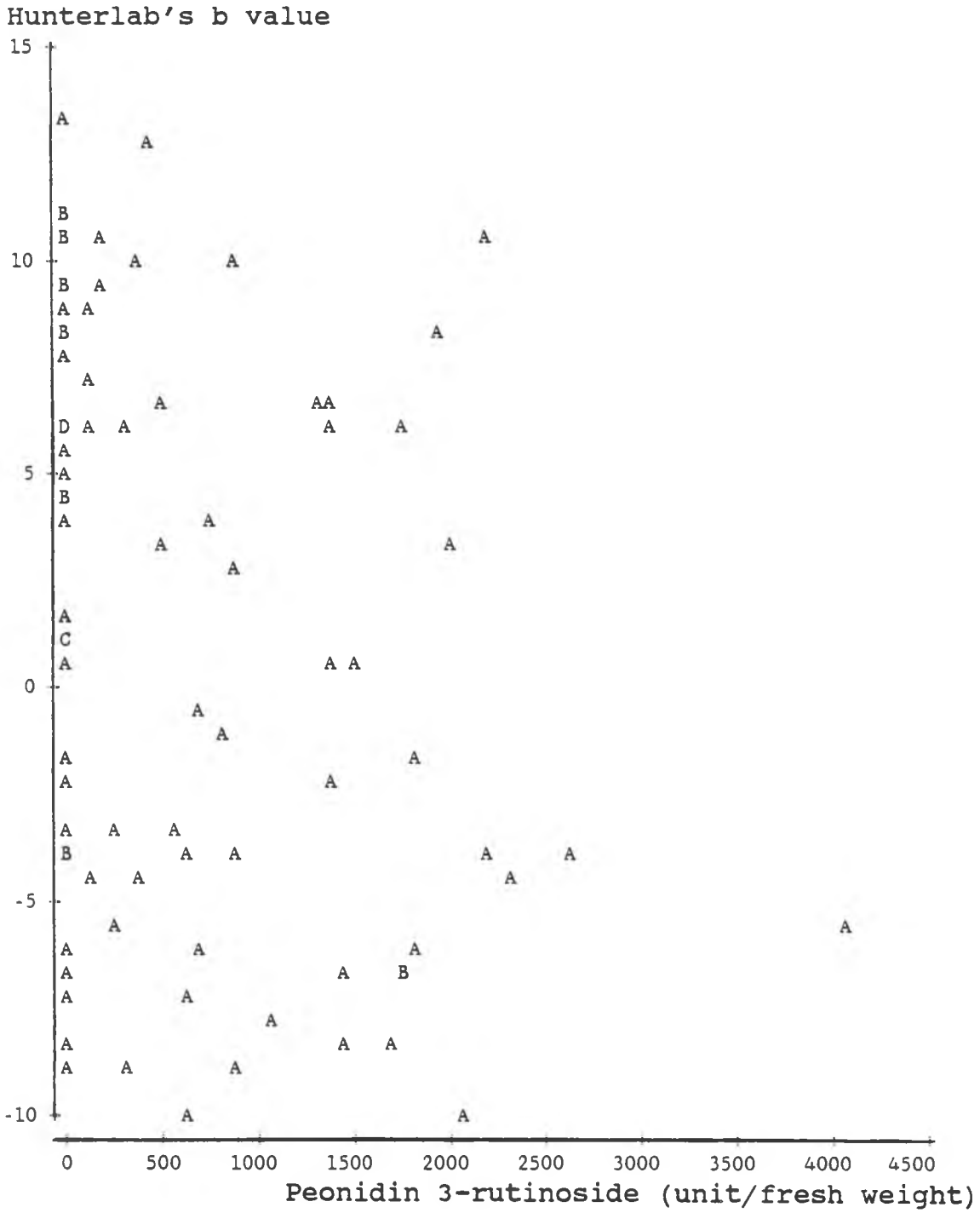

Figure A2. The distribution of peonidin 3-rutinoside in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

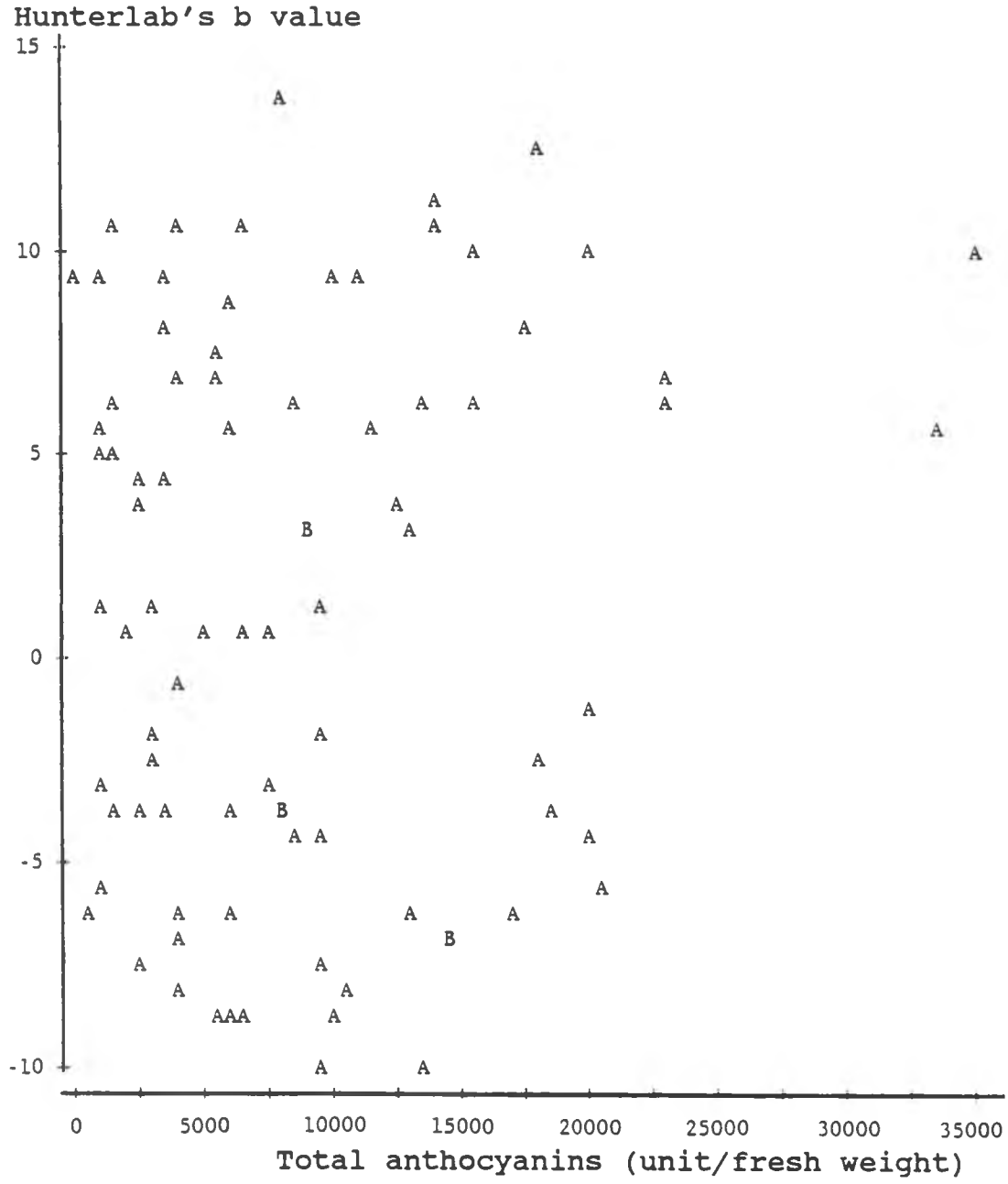

Figure A3. The distribution of total anthocyanins in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

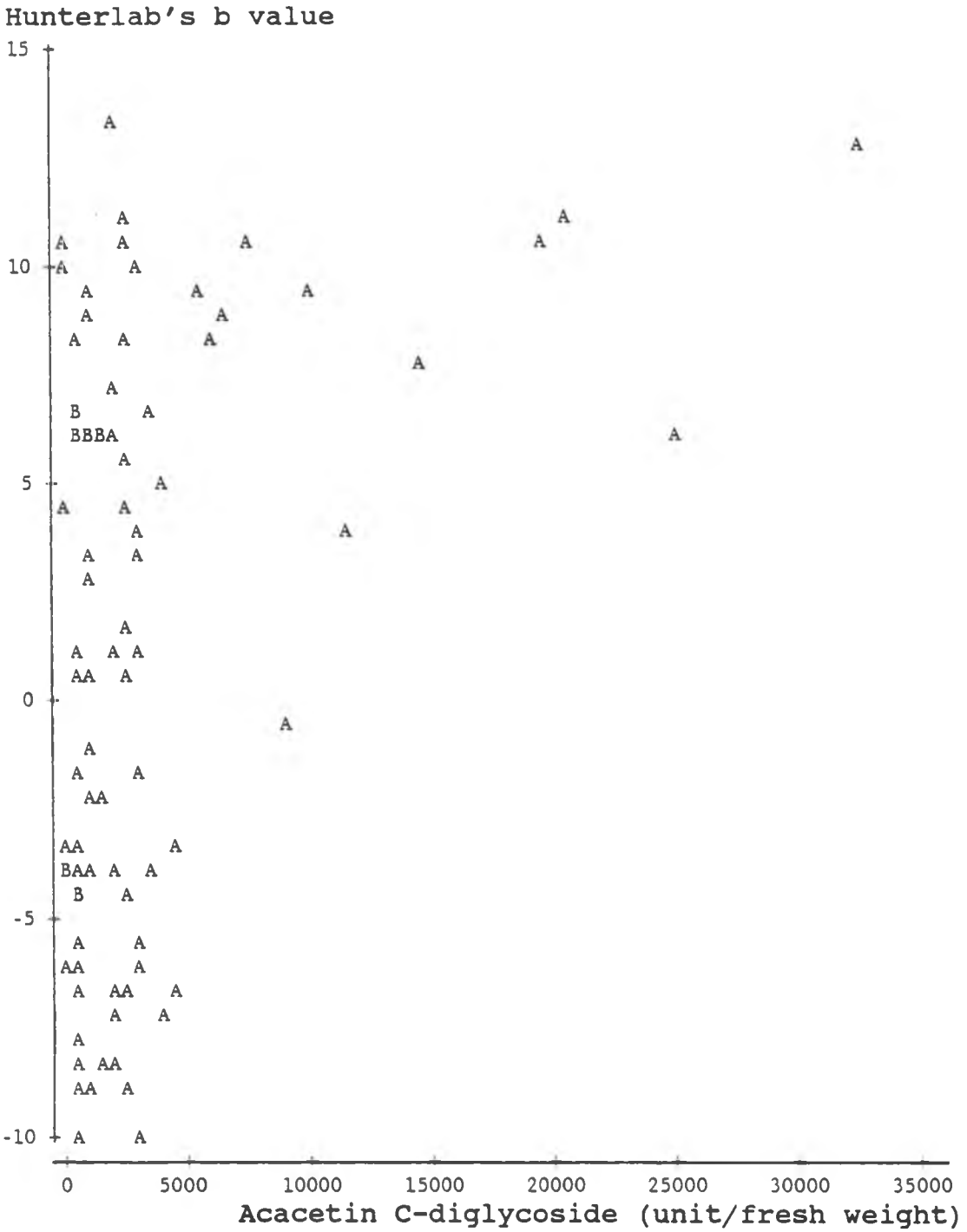

Figure A4. The distribution of acacetin C-diglycoside in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

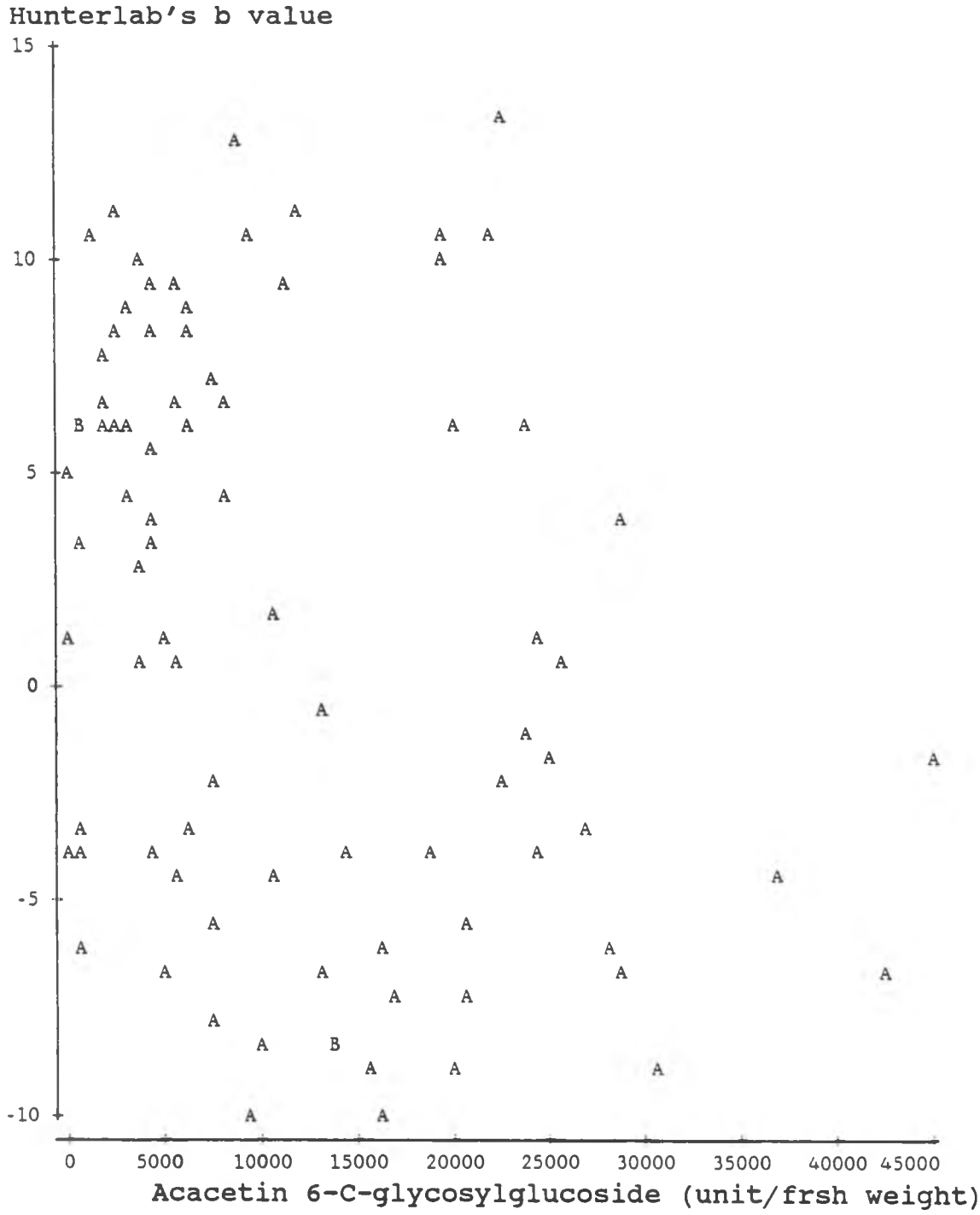

Figure A5. The distribution of acacetin 6-C-glycosyl-glucoside in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

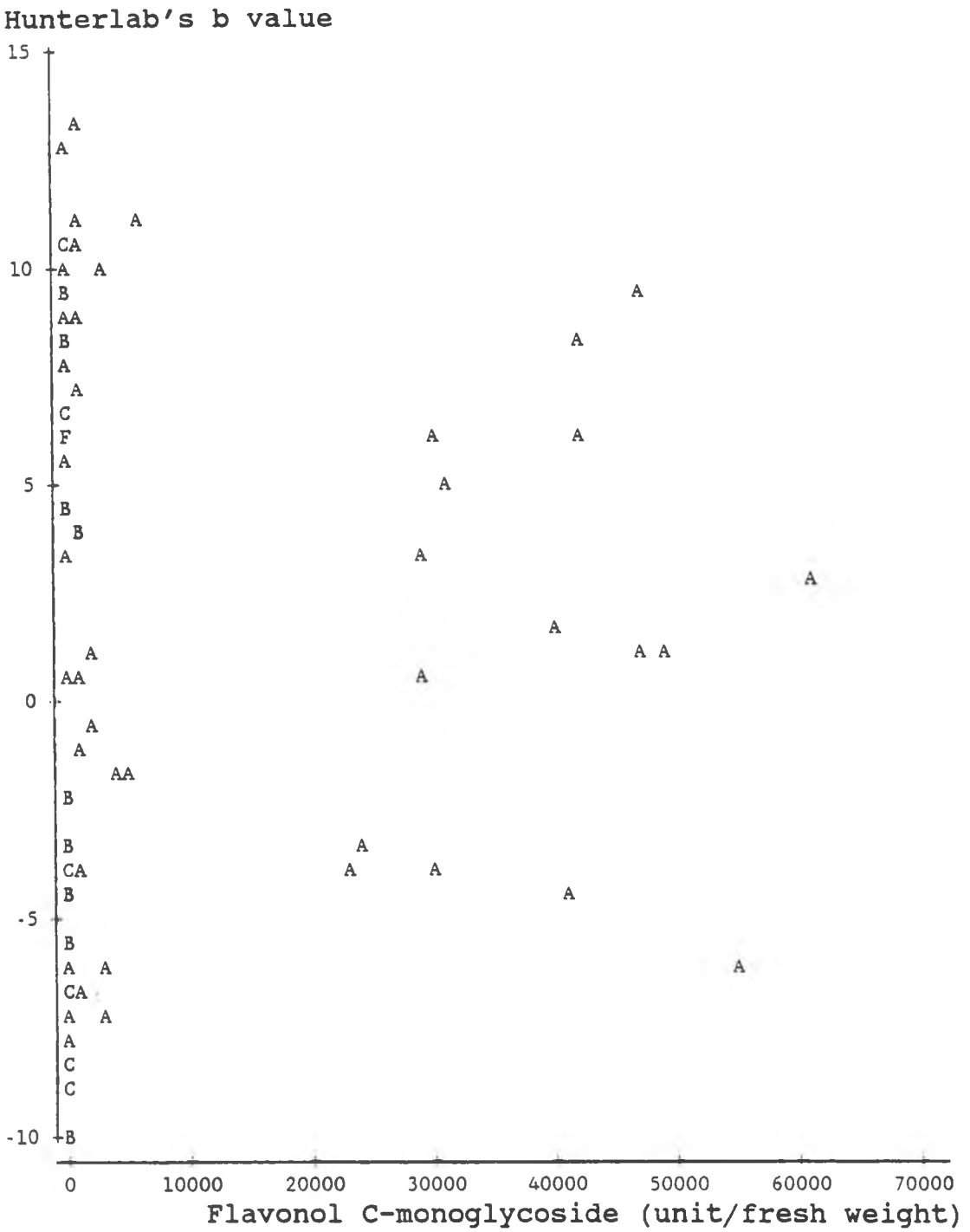

Figure A6. The distribution of flavonol C-monoglycoside in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

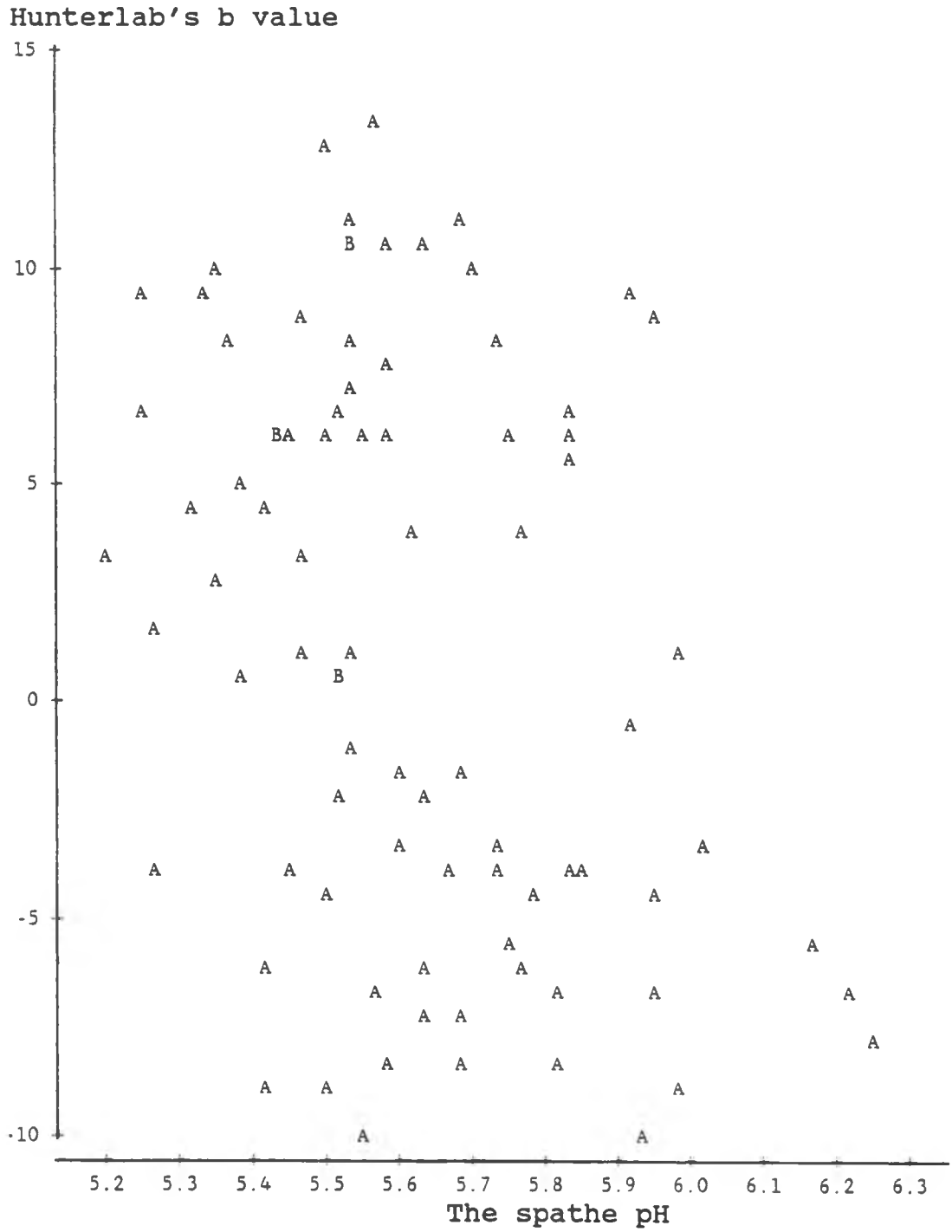

Figure A7. The distribution of the spathe pH in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

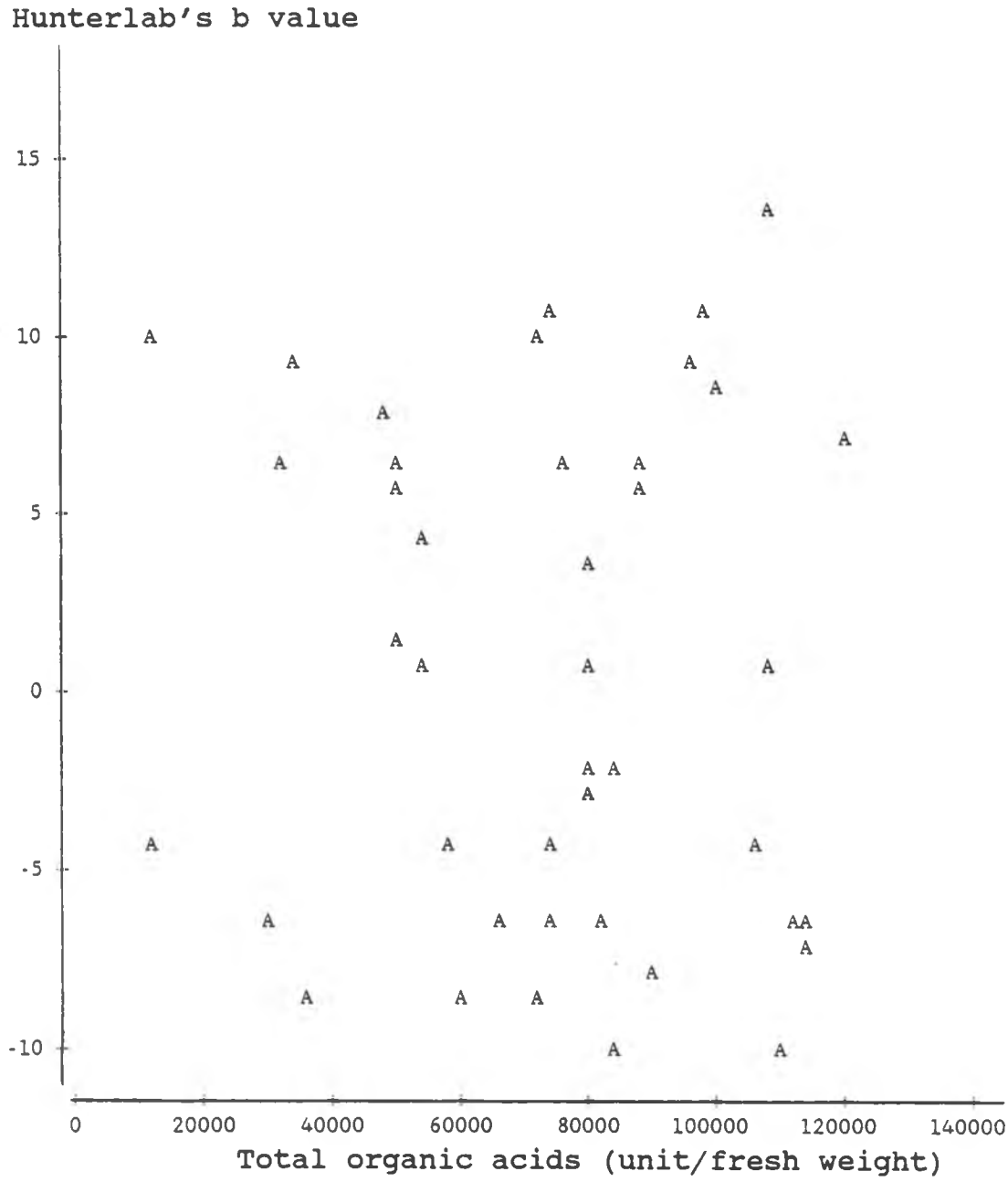

Figure A8. The distribution of total organic acid in cross 587.
A=1 observation, B=2 observations, C=3 observations.

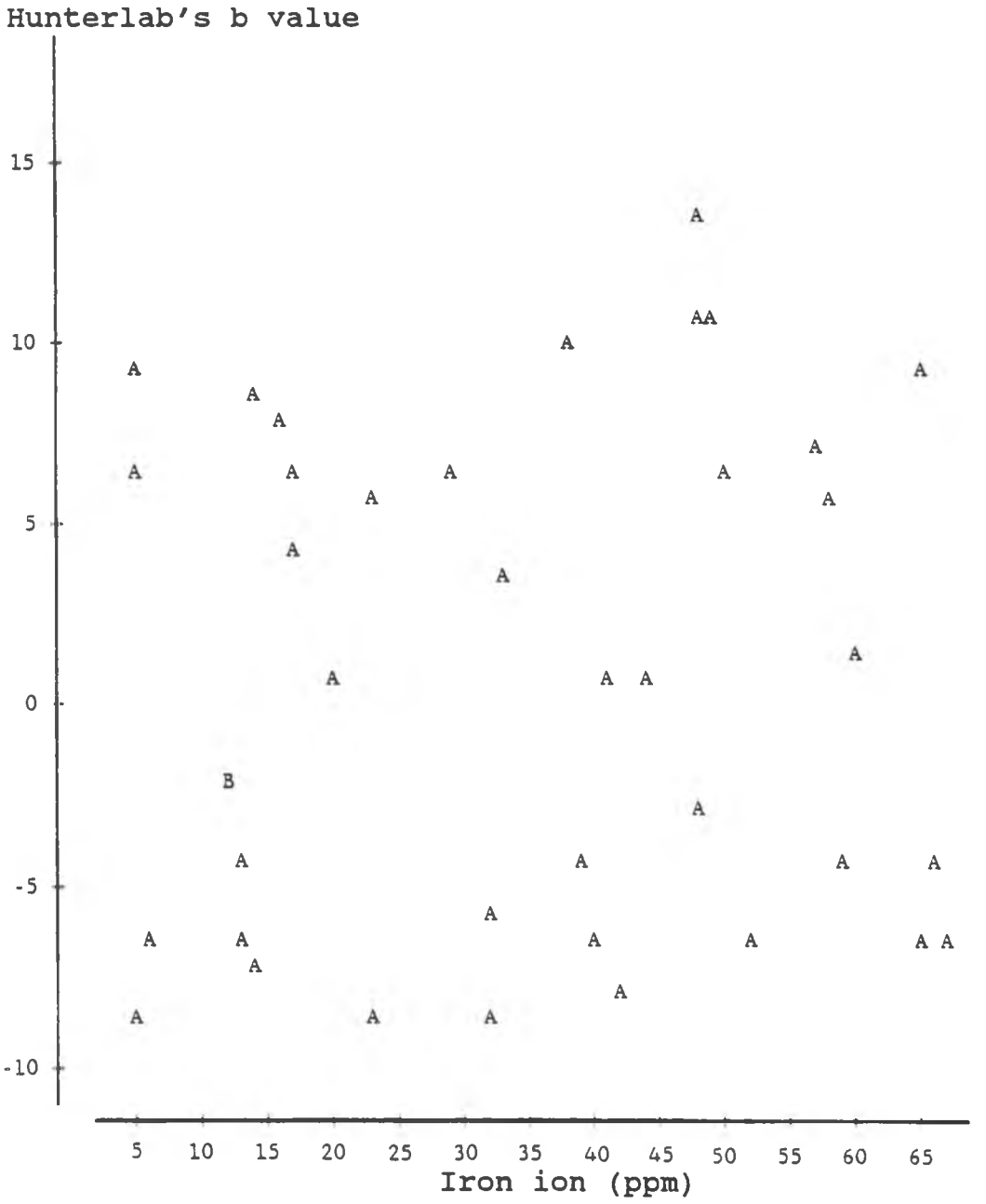

Figure A9. The distribution of iron ion in cross 587. A=1 observation, B=2 observations, C=3 observations.

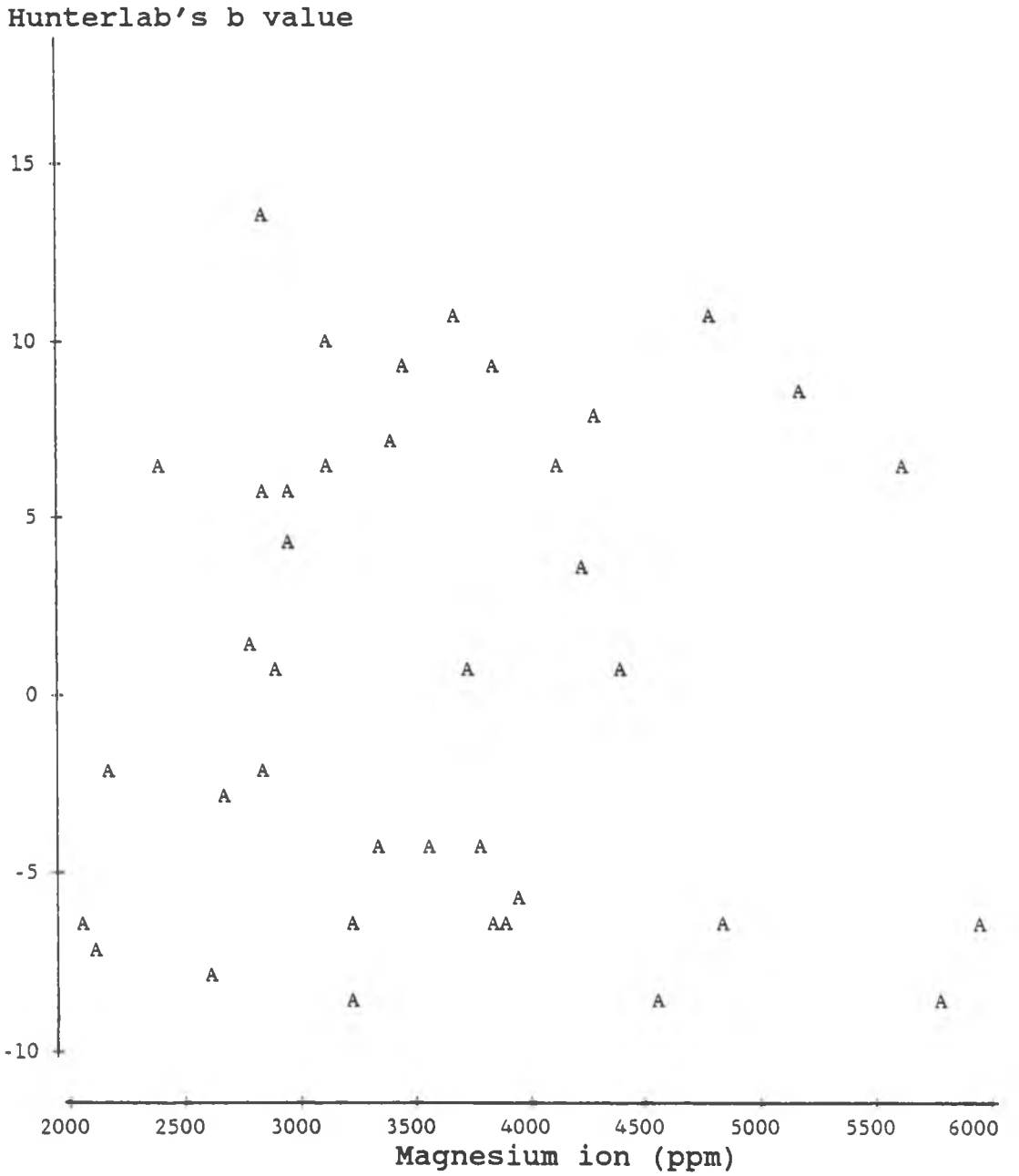

Figure A10. The distribution of magnesium ion in cross 587.
A=1 observation, B=2 observations, C=3 observations.

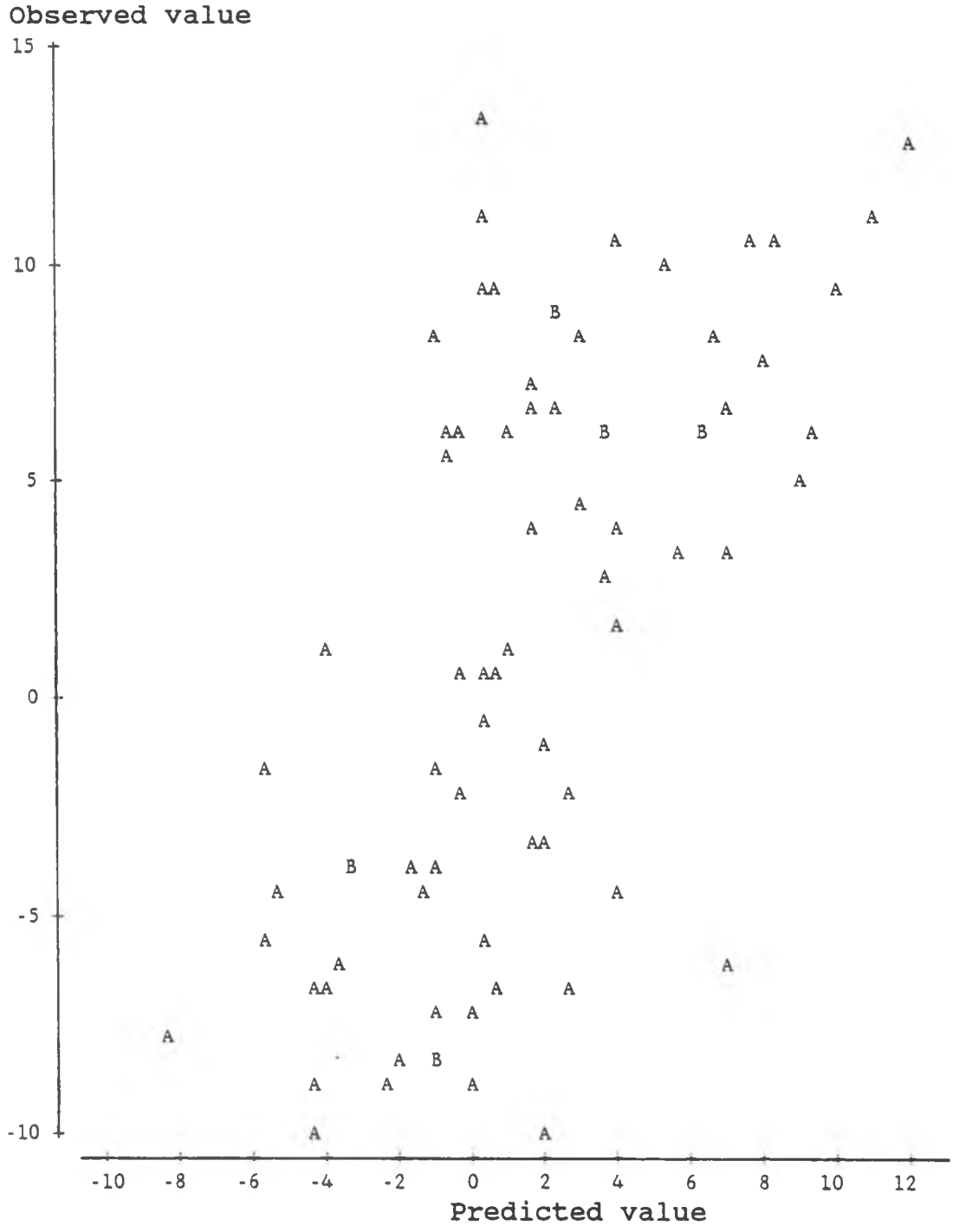

Figure A11. The distribution of regression predicted Hunterlab's b values against the observed values in the six segregating crosses.
A=1 observation, B=2 observations, C=3 observations.

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